The thesis committee for Ashlee Danielle Simpson
certifies that this is the approved version of the following thesis:

Chronic and Acute Effects of Hydroxytyrosol on Antioxidant Status and Inflammation at Rest and During Exercise

APPROVED BY
SUPERVISING COMMITTEE:

Supervisor:

John L. Ivy

Roger P. Farrar

Chronic and Acute Effects of Hydroxytyrosol on Antioxidant Status and Inflammation at Rest and During Exercise

by

Ashlee Danielle Simpson, B.S.

Thesis

Presented to the Faculty of the Graduate School of

The University of Texas at Austin

in Partial Fulfillment

of the Requirements

for the Degree of

Master of Science in Kinesiology

The University of Texas at Austin

May 2012

Acknowledgements

I want to recognize and express my gratitude to those that made this project possible. First, Dr. Ivy: Thank you for your guidance, patience, and imparting your intellect throughout my graduate career. Dr. Farrar: Thank you for listening, motivating, and sharing your ideas. Lynne Kammer: Thank you for your practical approach, organizational skills, and sharing great music. Zhenphing Ding: Thank you for your kind words and wisdom in the lab. I give my sincerest appreciation to all the research assistants on this project. Each one of you played a vital role in completing this project: Marin Healy, David Lassiter, Yoolee Kwon, Heontae Kim, Yang Liu, Ben Dessard, Lisa Stegall, Wanyi Wang, Bei Wang, Chen Wang, Ming Hsieh, Jungyun Hwang, Joowon Lee, James Burns, Michael Rodriguez, David Pollard, Elizabeth Cantu, Shelby Bowden, Lauren Weeks, and Alison Garner. I give a special thanks to the participants in this study.

I also want to thank those who were not a part of this project. My undergraduate advisor, Dr. Meyers: Thank you for believing in me and encouraging me to reach above and beyond as well as to always "Begin with the end in mind." And last, but definitely not least, my family: Your love, encouragement, and support have allowed me to (finally) finish my thesis and graduate with the first master's degree of our family.

Ashlee D. Simpson

May 4, 2012

Abstract

Chronic and Acute Effects of Hydroxytyrosol on Antioxidant Status and Inflammation at Rest and During Exercise

Ashlee Danielle Simpson, M.S. KIN

The University of Texas at Austin, 2012

Supervisor: John L. Ivy

Evidence shows that consumption of a Mediterranean diet can lower the risk of all-cause and cause-specific mortality suggesting that this diet has an overall effect on health. Antioxidants found within olive oil, the primary source of fat in the Mediterranean diet, may be leading contributors to the decreased disease risk. More specifically, hydroxytyrosol (HT), one of the most active and powerful antioxidants found in olive oil, has the ability to increase total antioxidant status and lower levels of lipid peroxidation. In addition to a healthy diet, physical activity decreases the risk of cardiovascular morbidity and mortality; however, aerobic exercise of sufficient intensity or duration can induce oxidative stress. Therefore, the purpose of this study was to investigate the effects of 6 weeks of HT supplementation on antioxidant status and markers of inflammation in healthy, recreationally active males before and throughout acute aerobic exercise bouts. Using a randomized, double-blind, repeated-measures, placebo-controlled design, sixty-one (n=61) participants were randomly assigned to

consume a placebo (PLA), low dose of HT (LHT, 50 mg/day), or high dose of HT (HHT, 150 mg/day). Throughout the course of the study, the participants performed four time trial rides (TT1-TT4) on cycle ergometers. TT1 occurred before supplementation, TT2 halfway through the supplementation period, and TT3 and TT4 occurred in the sixth week and final two days of supplementation. Blood was drawn prior to (pre) and just before termination (end) of each time trial to measure markers of antioxidant status and inflammation during exercise. We did not observe significant main effects for treatment on any of the markers for antioxidant status (TEAC) or for markers of inflammation (oxLDL, CRP, 8IP, TNFα, IL-6, IL-10, IL-1β, or IL-1ra). Significant treatment-by-time interactions occurred for CRP, 8IP, and IL-6 although significant treatment differences in these measures were not detected. We conclude that chronic and acute HT supplementation does not improve antioxidant status nor decrease markers of inflammation in this population at rest, during, or following exercise.

Keywords: hydroxytyrosol, oxidative stress, aerobic exercise, inflammation, antioxidant

Table of Contents

INTRODUCTION

Cardiovascular disease is one of the leading causes of death in industrialized nations and the second leading cause of death worldwide (Patrick & Uzick, 2001). However, epidemiological studies show that persons who consume a Mediterranean diet have a lower risk of all-cause and cause-specific mortality suggesting that this diet has an effect on overall health (Bogani et al., 2007; Carluccio et al., 2003; Cicerale et al., 2010; Covas, 2007; Minich & Bland, 2008; Pellegrini et al., 2003; Psaltopoulou et al., 2004). Olive oil is the primary source of fat in the Mediterranean diet and is associated with lower incidences of coronary heart disease and other degenerative diseases (Covas, 2007; Pellegrini et al., 2003). The antioxidants found in olive oil may be a primary contributor to decreased disease markers. For instance, they have the ability to decrease plasma levels of oxidized LDL and improve oxidative stress markers including lipid hydroperoxides, malondialdehyde, and activity of endogenous antioxidant defense systems (Raederstorff, 2009; Liu et al., 2009). More specifically, hydroxytyrosol (HT), one of the most active and powerful antioxidants found in olive oil, has the ability to increase total antioxidant status in rabbits and lower levels of isoprostanes and oxidized LDL, both markers of lipid peroxidation, in humans (Gonzalez-Santiago et al., 2006; Mastaloudis et al., 2001; Raederstorff, 2009; Vazquez-Velasco et al., 2010; Vincent & Taylor, 2006).

In conjunction with a healthy diet, physical activity is also associated with a decreased risk of cardiovascular morbidity and mortality (Mataix et al., 2008). Despite the associated health benefits of exercise, sufficient intensity or duration of physical

activity can generate reactive oxygen species, which can increase oxidative stress often associated with an inflammatory state (Teixeira et al., 2009). Recent research has focused on the effects of quercetin, an antioxidant found in the skins of many common fruits and vegetables, and its possible role in decreasing exercise-related muscular damage and inflammation. Although equivocal results have been found with quercetin supplementation, to-date little research exists on HT consumption in conjunction with aerobic exercise.

Therefore the purpose of this study was to: 1) observe changes in total antioxidant status and markers of inflammation in human blood with regular daily HT supplementation; 2) observe effects of an acute aerobic exercise bout on antioxidant status and markers of inflammation with regular daily HT supplementation; 3) compare changes throughout the acute aerobic exercise bout with regular daily HT supplementation; and 4) determine if changes in antioxidant status and markers of inflammation were greater with a high dose of HT (150 mg/day) when compared to a low dose (50 mg/day).

METHODS

Experimental Design

This study was a randomized, double-blind, repeated-measures placebo-controlled study. The 61 participants were randomized into one of three groups for hydroxytyrosol (HT) supplementation: placebo (PLA), low dose (LHT) of HT (50 mg/day), or high dose (HHT) of HT (150 mg/day). A third party, Metronomia (Muenchen, Germany) performed the randomization to preserve researcher and participant blinding. The algorithm attempted to evenly distribute the body weights of the participants in each supplementation group. Participants entering the study and weighing less than 80 kg were assigned the lowest available study product number, and those participants entering the study and weighing greater than or equal to 80 kg were assigned the highest available study product number.

Throughout the course of the nine-week study, the participants performed four time trial visits at the Exercise Physiology and Metabolism (EPM) Laboratory, Bellmont Hall, The University of Texas at Austin. Each time trial consisted of a 20 km simulated bicycle ride on a stationary cycle-ergometer (Velotron RacerMate, Inc., Seattle, WA). The baseline time trial (TT1) was performed before study product distribution to the participant. Participants performed a mid-point time trial (TT2) after three weeks of supplementation, and in the sixth week two consecutive time trials (TT3 and TT4, respectively) were performed on the final two days of supplementation.

At each time trial, venous blood was drawn two times throughout the visit from an antecubital vein using a catheter to obtain whole blood samples for measurement of antioxidant status and inflammatory markers. The first blood draw was taken 30 minutes before exercise commenced (pre) and another blood draw immediately before the very end of the time trial course (end). At TT2, TT3, and TT4, the pre blood draw was taken 30 minutes after ingestion of the study product just before commencement of exercise. Measurements of antioxidant status and inflammatory markers were compared for each participant during each time trial visit. The pre, or resting, measurements and changes during exercise were assessed across the length of the study for TT1, TT2, and TT3 to determine chronic effects of the study product. Resting measurements and changes during exercise were also assessed between TT3 and TT4 to determine acute effects of the study product. Antioxidant status and markers of inflammation were also contrasted against the measurements of the three different supplementation groups. Measures of antioxidant status included: Trolox Equivalent Antioxidant Capacity (TEAC) and uric acid. Measures of inflammation included: oxidized low-density lipoprotein (oxLDL), C-reactive protein (CRP), 8-isoprostane (8IP), and cytokines (tumor necrosis factor alpha (TNFα), interleukin 6 (IL-6), interleukin 10 (IL-10), interleukin 1-beta (IL-1β), and interleukin 1-receptor antagonist (IL-1ra)).

Participants

Prior to recruitment of participants, the study protocol and the *Informed Consent to Participate in Research* (Appendix A) was approved by the Institutional Review Board at The University of Texas at Austin. Participants were recruited through posters placed

around the campus of The University of Texas at Austin and through the "Hire a Longhorn" website, an Internet-based classified ads system available to UT students. Qualification for recruitment included healthy participants who were recreationally active males between 20 and 35 years of age. Those willing to participate contacted the study's investigators at the EPM Laboratory via telephone and/or email to express interest in participation. Participants were then scheduled for a screening visit.

Study Supplement

The study supplement was manufactured and distributed by DSM Nutritional Products (Kaiseraugst, Switzerland). The wastewater produced by olive oil manufacturing was utilized for extraction of HT as this wastewater contains a considerable amount of phenols that are washed off during the olive oil production process (Visioli & Galli, 1998). The HT extracted from this process was placed into capsules containing 50 mg HT. Participants were instructed to consume one packet each day and each packet contained three capsules of study product. The PLA dose capsules contained only modified starch. The LHT dose packet contained one capsule with 50 mg HT and two capsules with modified starch. The HHT dose packet contained three capsules with 50 mg HT each, thus a total of 150 mg of HT.

After the baseline time trial, participants were assigned a study product number based on weight as previously described and given a two-week supply. The study product was consumed for a total of six-weeks. Participants were instructed to keep the study product refrigerated at all times and to consume one packet (3 capsules) of study product each morning with breakfast. If it was after 2:00 PM, the participant was

instructed to skip that day's dose and continue study product consumption the following morning. Participants were instructed on compliance with consumption of the study product. Compliance had to remain at or above 85% throughout the study period; therefore, participants could not skip more than one dose per week and had to consume 36 of the 42 total dosages. Skipped doses were to remain in the original packet and all packets, empty and full, as well as boxes were to be returned to the EPM Laboratory at the next appointment. All participants met the minimum compliance level and the remaining study product was returned to DSM Nutritional Products.

Dietary and Exercise Regimen

The participants in this study were instructed to maintain dietary logs (Appendix C) for 48 hours prior to each time trial and to avoid foods and beverages high in antioxidants. Foods and beverages consumed before the first time trial were to be mimicked before the subsequent time trials to limit dietary influences. Throughout the study period, participants were instructed to limit olive oil consumption to one tablespoon per day, coffee or tea to one cup per day, wine to two glasses per week, and chocolate bars to two per week.

In the 24 hours prior to the time trial, participants were instructed to completely avoid the following foods as they contain a large amount of antioxidants that could interfere with interpretation of the study product's effects: coffee, tea, wine, chocolate, cherry tomatoes, broccoli, blueberries, and onions. In this study, participants were given one bottle of vanilla-flavored Ensure® (Abbott Nutrition, Cleveland, OH), provided by the staff of the EPM Laboratory, which was to be consumed 10 hours before the

appointment time. At this time, fasting commenced except for water and hydration maintenance was encouraged.

Participants were instructed to maintain recreational activities throughout the course of the study, and strenuous exercise was to be avoided in the 24 hours prior to the time trials and safety blood draws.

Participants recorded time of supplement consumption, physical activity, and overall physical and emotional wellbeing on a daily online log (Zoomerang, San Francisco, CA). EPM Laboratory staff alerted participants via text message or phone call if the log was not completed by 6:00 PM each day.

Health and Safety Procedures

An initial blood draw at the familiarization visit was performed to test for Hepatitis B and C. Four milliliters of whole blood were drawn from an antecubital vein under sterile conditions using a Vacutainer® (BD, Franklin Lakes, NJ) serum separator tube at the familiarization visit. Blood was allowed to mix for 15 minutes and then the tube was centrifuged at 1300 g for 15 minutes. The tube was then stored in a biological hazard safety bag in a four-degree Fahrenheit refrigerator until picked up by personnel from an outside laboratory for testing (Quest Diagnostics, Irving, TX). Test results were available online and reviewed by the study physician and EPM Laboratory staff before the subsequent visit. No participants in this study tested positive for Hepatitis B or C.

Upon arrival to the EPM Laboratory for every visit, participants were asked if adverse events, including sickness and/or trauma, had occurred since the last visit. These were documented by laboratory staff and reviewed by the study physician to determine if

related to the study product. If the study physician deemed the adverse event a result of the study product and endangered the participant, the participant was excused from the study and recommended to visit his primary care physician or the study physician. However, no adverse events excused participants from this study.

Test Administration

SCREENING VISIT

The screening visit took place at the Fitness Institute of Texas (FIT), Bellmont Hall, at The University of Texas at Austin. During this visit, the participants read and signed the *Informed Consent to Participate in Research*. Next, participants were asked to complete a *Participation Health Research Screening Form* (Appendix B) to determine inclusion and exclusion criteria. If the participant answered "no" to questions regarding a medical history inclusive of kidney, liver, or cardiovascular conditions, then he was asked to fully participate in the study. Medications taken by the participant for allergies, depression, and/or thyroid hormone imbalances were acceptable only if the dosage had been consistent for the past two months. Medications for treatment of cardiac, lipid, or hypertensive conditions, or blood-glucose regulators excluded the participant from the study.

If the participant met the criteria, then study investigators measured the participant's height using a stadiometer, weight using a scale, and blood pressure using a sphygmomanometer. Height and weight were used to assess body mass index (BMI). Participants were only asked to partake in the study if BMI was less than or equal to 30 kg/m^2 and if his blood pressure was less than or equal to 140 mmHg systolic over 90

mmHg diastolic. If all criteria were met, then the remaining study visits were scheduled including one familiarization visit with Hepatitis B and C blood draw screening and four time trial (TT) visits.

FAMILIARIZATION RIDE

The purpose of this visit was to acquaint the participant with the cycle ergometer and computer-based time trial course. This visit took place approximately one week prior to the baseline time trial. The participant was instructed to fast for two hours prior to the appointment time and arrive at the EPM Laboratory in loose, comfortable clothing and athletic shoes. Upon arrival, he was weighed and his weight recorded. He was then fitted with a heart rate monitor (Polar Electro Inc., Lake Success, NY), and his resting heart rate was recorded after five minutes of sitting quietly. Blood was drawn to check for Hepatitis B and C. After the blood draw, the participant mounted the stationary Velotron cycle ergometer (RacerMate Inc., Seattle, WA) and adjustments for a comfortable fit were made and recorded. The Velotron cycle-ergometer and compatible Computrainer software (RacerMate Inc., Seattle, WA) installed on Hewlett-Packard computer systems (Palo Alto, CA) was used for this study because the data extracted from use of the Velotron cycle ergometers is highly reproducible (Sporer & McKenzie, 2007). The participant was instructed to perform the 20 km time trial ride as fast as possible. Heart rate and rating of perceived exertion (RPE) using the Borg scale were measured and recorded halfway through and right before the very end of the time trial course. Water was provided to the participant *ad libitum*. Upon completion of the time trial course, the participant performed a cool down until his heart rate reached 120 bpm or

less. Then he dismounted from the cycle ergometer and returned the heart rate monitor to the EPM Laboratory staff. Instructions for the baseline time trial were given to the participant.

TIME TRIALS (TT)

One week after the familiarization ride, participants performed the baseline time trial ride (TT1). Upon arrival, the EPM Laboratory staff asked the participant about his exercise, food, and study product compliance and recorded this in the participant's binder. Next a 20 gauge Teflon catheter, fitted with a three-way stopcock, was inserted into an antecubital vein under sterile conditions to draw blood. The catheter was fitted with extension tubes and taped securely in place so that successive blood draws could easily be made throughout the visit while the participant was riding the cycle ergometer. A pre-exercise (pre) blood draw was performed prior to commencement of exercise. A 10 ml syringe was fitted to the end of the three-way stopcock, the stopcock was turned so that blood flowed into the syringe with suction, and filled completely with whole blood. Blood was drawn following this same procedure right before completion (end) of the time trial course.

Five-milliliters of this blood was distributed between two 12 X 75 mm culture test tubes (Fisherbrand, Hampton, NH) of which 2 ml were distributed into a tube containing 200 µl of ethylenediaminetetraacetic acid (EDTA) (24 mg/ml, pH 7.4) and 10 µM indomethacin, and the remaining 3 ml were distributed into a tube containing 200 µl of EDTA only.

The remaining 5 ml of blood were transferred to a Vacutainer® serum-separator tube (SST). The collection tubes and SST were inverted five times and left on ice for approximately 40 minutes. After incubation the three collection tubes were centrifuged at 3000 rpm for 10 minutes using an HS-4 rotor in a Sorvall RC6 centrifuge (Kendro Laboratory Products, Newtown, CT), and the SST was centrifuged at 1300 g for 15 minutes with an IEC Cl31R Multispeed Centrifuge (Thermo Electron Corporation, Waltham, MA). After centrifugation, the plasma and serum supernatant was extracted off the top and divided into the respective microcentrifuge storage tubes. Twenty microliters of butylated hydroxyl toluene was added immediately before storage to the tube labeled 8-Isoprostane for assay purposes. These tubes were stored in a -20°C freezer until set and then placed in a -80°C freezer until further analysis.

After completion of the time trial, the participant cooled down until his heart rate dropped to 120 bpm or less. He then sat in a chair while EPM Laboratory staff removed the catheter, cleaned the area with an alcohol swab, and then placed a cotton ball and bandage over the puncture site. The participant was then provided a light snack.

After TT1, the participant was then randomized based on his weight and two-weeks of study product were distributed to him. Verbal and written instructions were provided to the participant regarding expectations of the study and consumption of the study product.

Test administration for the subsequent trials was repeated for TT2, after 21 days of supplementation, and TT3 and TT4, on days 41 and 42 of supplementation, respectively. At these visits, the participant took the study product 30 minutes prior to

exercise commencement under the supervision of EPM Laboratory personnel. TT3 and TT4 were performed on consecutive days in order to observe effects of an increased inflammatory response and any acute treatment effects of the study product.

BLOOD TISSUE ANALYSIS

Antioxidant Status. Antioxidant status was measured in serum using Trolox Equivalent Antioxidant Capacity (TEAC) (Cayman Catalog No. 709001, Ann Arbor, MI) The kit has an intra-assay percent coefficient of variation (%CV) equal to 3.40% and inter-assay %CV equal to 3.00%. The capacity of the antioxidants in the serum sample to prevent oxidation of ABTS® (2,2'-azinobis-3-ethylbenzothiazoline-6-sulphonic acid) is measured relative to Trolox. A value is then assigned to the degree of discoloration yielding the TEAC measure (Morillas-Ruiz et al., 2006). Uric acid was converted from mg/dl to mM and subtracted from TEAC to yield adjusted TEAC.

Uric Acid. Uric Acid was measured in serum by enzymatic determination using Uric Acid Reagent (Raichem, Cliniqa, CA). The kit has an intra-assay %CV equal to 0.89% and inter-assay %CV equal to 1.00%.

Oxidized Low-Density Lipoprotein. Oxidized low-density lipoprotein (oxLDL) in blood serum was allowed to bind to $\beta_2$glucoprotein-I to form an oxLDL-β_{2G}GPI complex, then measured using an Elisa sandwich assay (Cayman EIA Catalog No. 10007893, Ann Arbor, MI). The kit has an intra-assay %CV equal to 4.80% and inter-assay %CV equal to 10.00%. Results of this assay were calculated using MasterPlex® ReaderFit: Curve-Fitting Software for ELISA Analysis (Hitachi Solutions America, South San Francisco, CA).

C-Reactive Protein. C-reactive protein (CRP) was measured using an immunoturbidimetric assay to assess antibody and antigen immunoprecipitation complexes found in blood serum (Raichem, Catalog No. 87545). The kit has an intra-assay %CV equal to 4.10% and inter-assay %CV equal to 3.20%.

8-Isoprostane. Levels of 8-isoprostane (8IP), which indicates oxidation of tissue phospholipids by reactive oxygen species (ROS) in serum samples, were measured enzymatically at an absorbance of 420 nm (Cayman EIA Catalog No. 516351, Ann Arbor, MI). The kit has an intra-assay %CV equal to 11.70% and inter-assay %CV equal to 16.40%. Results of this assay were also calculated using MasterPlex® ReaderFit: Curve-Fitting Software for ELISA Analysis (Hitachi Solutions America, South San Francisco, CA).

Cytokines. Concentrations of various cytokines, inflammatory response molecules, in serum samples were assessed using Millipore High Sensitivity Multiplex Cytokine Assay kit (Millipore, Billerica, MA) with the Bio-Plex 200 multiplex suspension array system, using Luminex xMAP detection technology (Luminex Corp., Austin, TX). The cytokines analyzed include: TNFα, IL-6, IL-10, and IL-1β. IL-1ra was measured using Millipore Multiplex Cytokine Assay kit (Millipore, Billerica, MA). Results of this assay were calculated using MILLIPLEX Analyst Software (Millipore, Billerica, MA). Intra-assay %CV for IL-1ra, TNFα, IL-6, IL-10, and IL-1β were 4.60%, 3.49%, 3.51%, 3.31%, and 3.11%, respectively and inter-assay %CV were 6.00%, 3.78%, 4.48%, 11.84%, and 2.16%, respectively.

All assays were performed at the conclusion of the fielding; unblinding occurred after completion of all assays. Assay protocols were followed as described by the company with each participant's time point ran in duplicate. Intra-assay %CV for the assays ran in our lab was calculated by dividing the standard deviation by the mean of the duplicate measure. The %CV for the assay was then averaged together. This was the case for TEAC, Uric Acid, oxLDL, CRP, and 8IP; whereas, %CV for the cytokines was calculated by MILLIPLEX Analyst Software. The %CV in our lab for the assays are as follows: TEAC – 5.15%, Uric Acid – 1.66%, oxLDL – 7.35%, CRP – 6.47%, 8IP – 2.15%, IL-1ra – 1.21%, TNFα – 0.55%, IL-6 – 0.76%, IL-10 – 2.92%, and IL-1β – 0.60%

DATA ANALYSIS

All data were analyzed using PASW 18.0 for Windows (SPSS Inc., Chicago, IL). Descriptive statistics were performed and included: mean, standard deviation, standard error of the mean, skewness and kurtosis. All data except 8IP and TEAC were skewed, with skewness or kurtosis ≥ 1.5, and transformed using a log transformation before running comparative statistics. Comparisons were made between pre values of TT1, TT2, and TT3 to assess chronic effects of the study product at rest, and changes within time trials (end – pre) were also compared across TT1, TT2, and TT3 to determine chronic effects of the study product during exercise. Comparisons were made between pre values of TT3 and TT4 to observe acute treatment effects of the study product at rest, and changes within time trials (end – pre) were also compared between TT3 and TT4 to determine acute effects of the study product on consecutive days of exercise. Data were analyzed both between and within treatments using repeated-measures ANOVA by

treatment-by-time. Changes (Δ) between time trials were compared using a oneway ANOVA. Where significance was found, post hoc analyses using LSD with no correction for multiple comparisons were performed. Differences were considered significant at $p<.05$. Data is expressed as mean ± standard error of the mean (SE).

RESULTS

Chronic effects of the study product on antioxidant status and markers of inflammation were examined by treatment between TT1, TT2, and TT3. Treatments included: placebo (PLA), low dose HT (LHT), and high dose HT (HHT). TT1 occurred before distribution of the study product (day 0), TT2 after 21 days of supplementation, and TT3 after 41 days of supplementation. Measurements of antioxidant status and markers of inflammation during each time trial were taken at rest (pre) and just before completion of exercise (end). Comparisons were made between pre values of TT1, TT2, and TT3 to assess chronic effects of the study product at rest, and changes within time trials (end – pre) were also compared across TT1, TT2, and TT3 to determine chronic effects of the study product during exercise.

Acute effects of the study product on antioxidant status and markers of inflammation were examined by treatment between TT3 and TT4. TT4 occurred 24 hours after TT3 (i.e. day 42 of supplementation). Comparisons were made between pre values of TT3 and TT4 to observe acute treatment effects of the study product at rest, and changes within time trials (end – pre) were also compared between TT3 and TT4 to determine acute effects of the study product on consecutive days of exercise.

Data was analyzed both between and within treatments using repeated-measures ANOVA by treatment-by-time. Changes (Δ) between time trials were compared using a oneway ANOVA. Where significance was found, post hoc analyses using LSD with no correction for multiple comparisons were performed.

TEAC

There was no significant chronic treatment effect or treatment-by-time interaction between TT1, TT2, and TT3 at rest or during exercise. Also, no significant acute treatment effects were found between TT3 and TT4 at rest or during exercise.

Table 1. *Descriptive data by time point and treatment for TEAC (mM).*

	PLA		LHT		HHT	
	PRE	END	PRE	END	PRE	END
TT1	1.03 ± 0.06	1.03 ± 0.06	0.92 ± 0.06	0.96 ± 0.05	0.96 ± 0.04	0.88 ± 0.06
TT2	1.08 ± 0.07	1.04 ± 0.08	0.99 ± 0.05	0.95 ± 0.04	1.01 ± 0.05	1.00 ± 0.04
TT3	0.95 ± 0.07	1.03 ± 0.07	0.89 ± 0.04	1.02 ± 0.06	0.93 ± 0.05	0.96 ± 0.03
TT4	1.06 ±0.08	1.03 ± 0.07	1.04 ± 0.05	1.02 ± 0.06	1.02 ± 0.05	1.03 ± 0.04

Values are means ± SE.

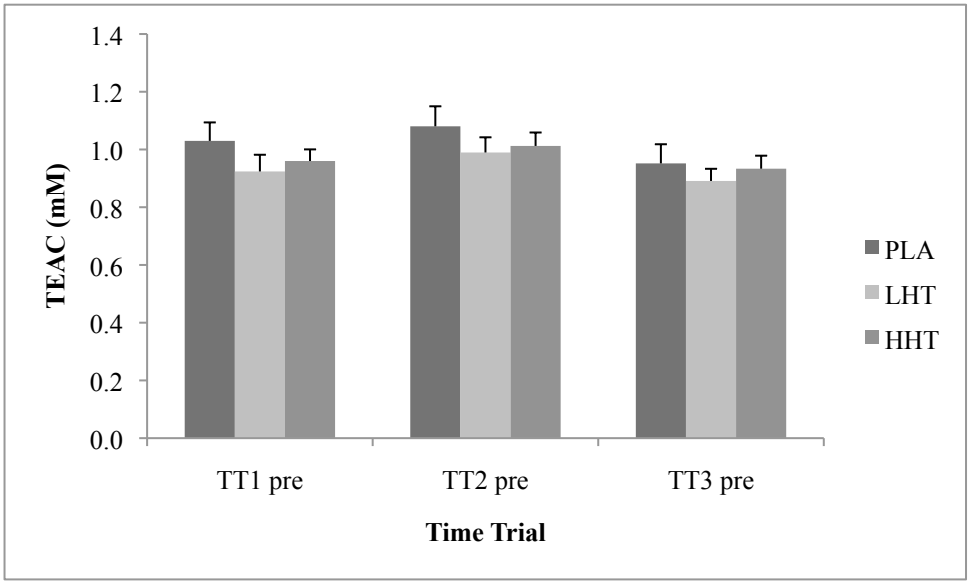

Figure 1.0. *Resting TEAC levels (mM) at TT1, TT2, and TT3 between treatments. Bars represent means ± S.E. No significant chronic treatment effect or treatment-by-time interaction was found.*

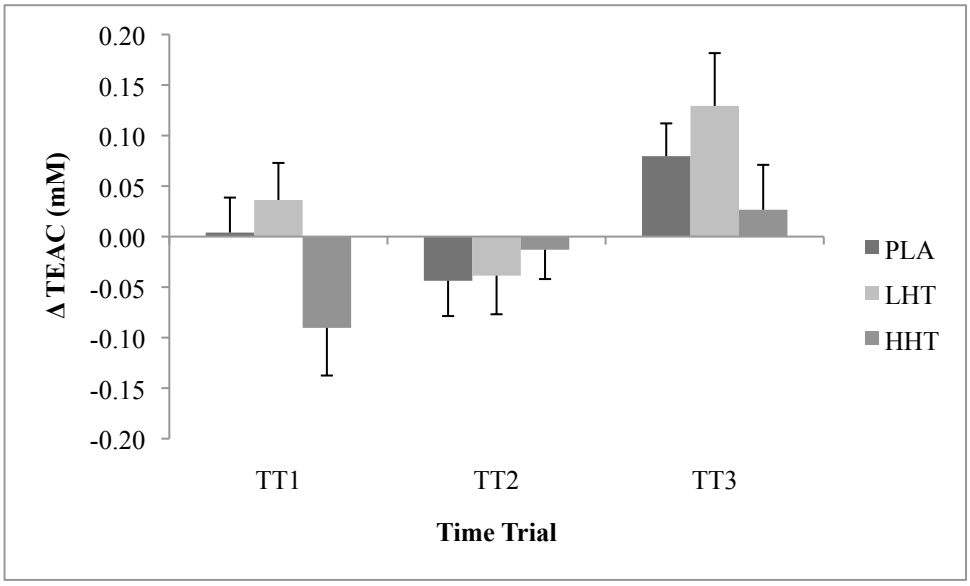

Figure 1.1. *Changes in TEAC (mM) during exercise at TTI, TT2, and TT3 between treatments. Bars represent means ± S.E. No significant chronic treatment effect or treatment-by-time interaction was found.*

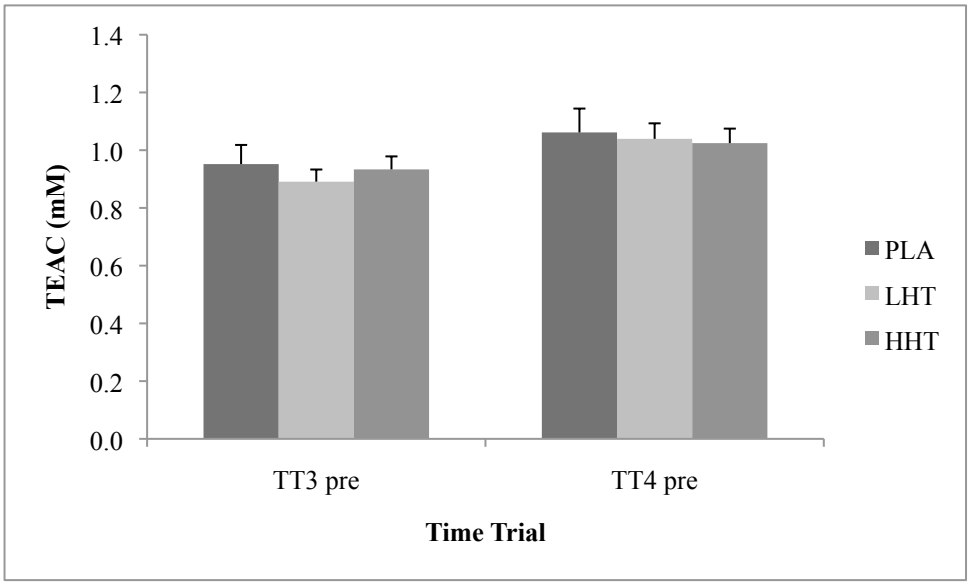

Figure 1.2. *Resting TEAC levels (mM) at TT3 and TT4 between treatments. Bars represent means ± S.E. No significant acute treatment effect was found.*

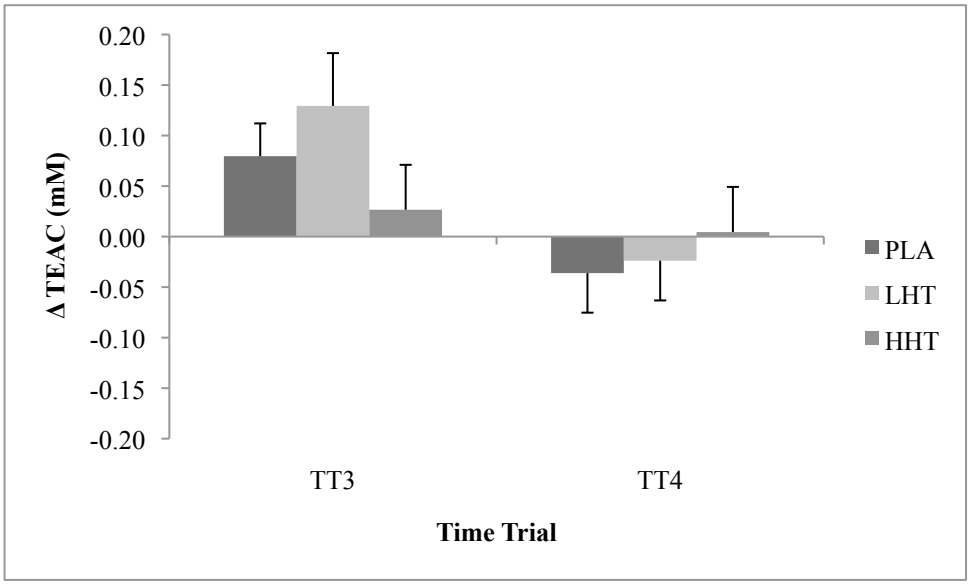

Figure 1.3. *Changes in TEAC (mM) during exercise at TT3 and TT4 between treatments. Bars represent means ± S.E. No significant acute treatment effect was found.*

oxLDL

There was no significant chronic treatment effect or treatment-by-time interaction between TT1, TT2, and TT3 at rest or during exercise. Also, no significant acute treatment effects were found between TT3 and TT4 at rest or during exercise.

Table 2. *Descriptive data by time point and treatment for oxLDL (ng/ml).*

	PLA		LHT		HHT	
	PRE	END	PRE	END	PRE	END
TT1	228.31 ± 100.47	289.36 ± 139.68	247.01 ± 98.95	218.93 ± 80.08	270.91 ± 122.94	308.02 ± 131.57
TT2	223.32 ± 106.39	270.40 ± 138.61	199.00 ± 89.83	226.78 ± 97.35	247.67 ± 96.96	281.44 ± 113.55
TT3	249.32 ± 145.04	262.09 ± 138.67	209.44 ± 90.85	240.76 ± 88.43	215.30 ± 79.56	243.41 ± 88.56
TT4	230.53 ± 131.00	246.15 ± 122.55	230.45 ± 98.99	277.89 ± 120.78	204.84 ± 72.72	232.06 ± 80.44

Values are means ± SE.

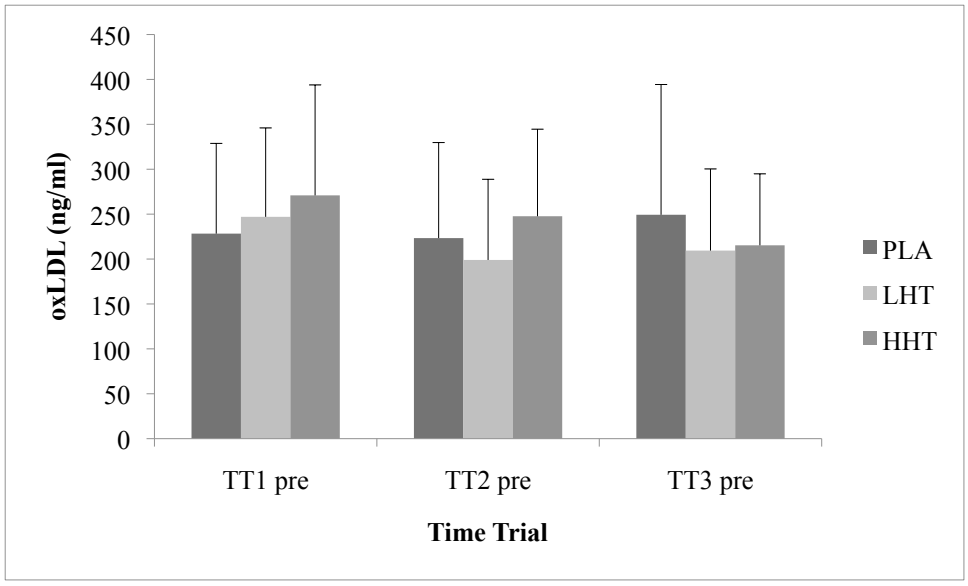

Figure 2.0. *Resting oxLDL levels (ng/ml) at TT1, TT2, and TT3 between treatments. Bars represent means ± S.E. No significant chronic treatment effect or treatment-by-time interaction was found.*

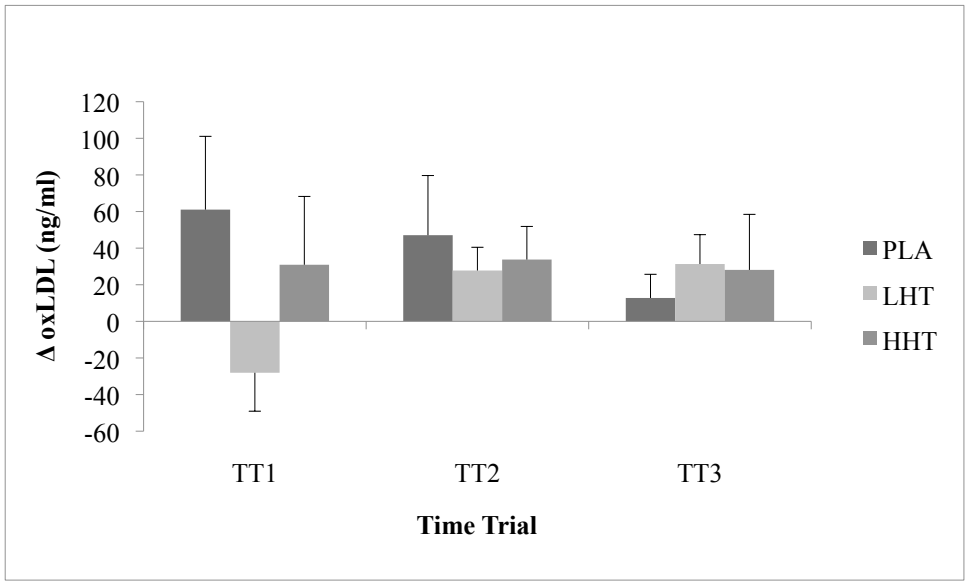

Figure 2.1. *Changes in oxLDL (ng/ml) during exercise at TTI, TT2, and TT3 between treatments. Bars represent means ± S.E. No significant chronic treatment effect or treatment-by-time interaction was found.*

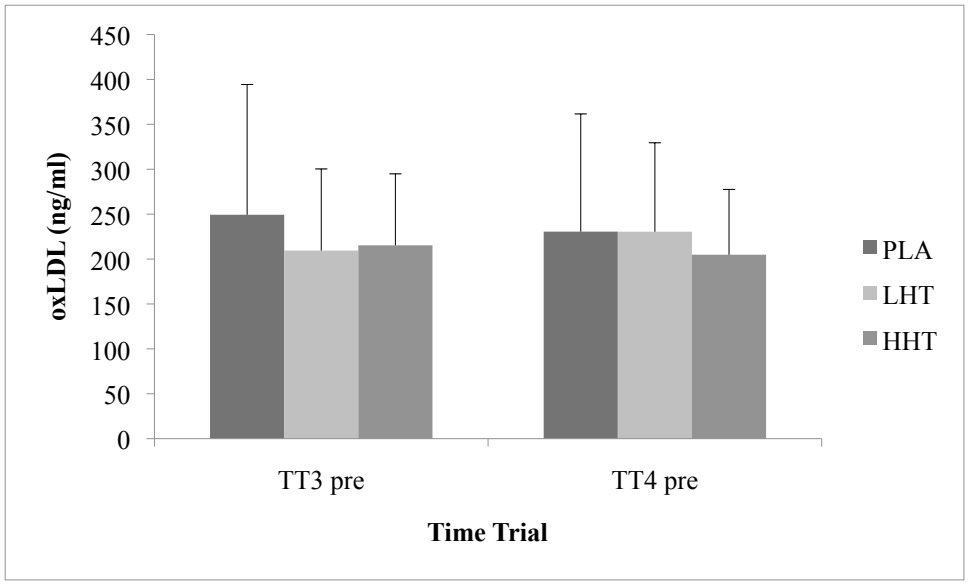

Figure 2.2. *Resting oxLDL levels (ng/ml) at TT3 and TT4 between treatments. Bars represent means ± S.E. No significant acute treatment effect was found.*

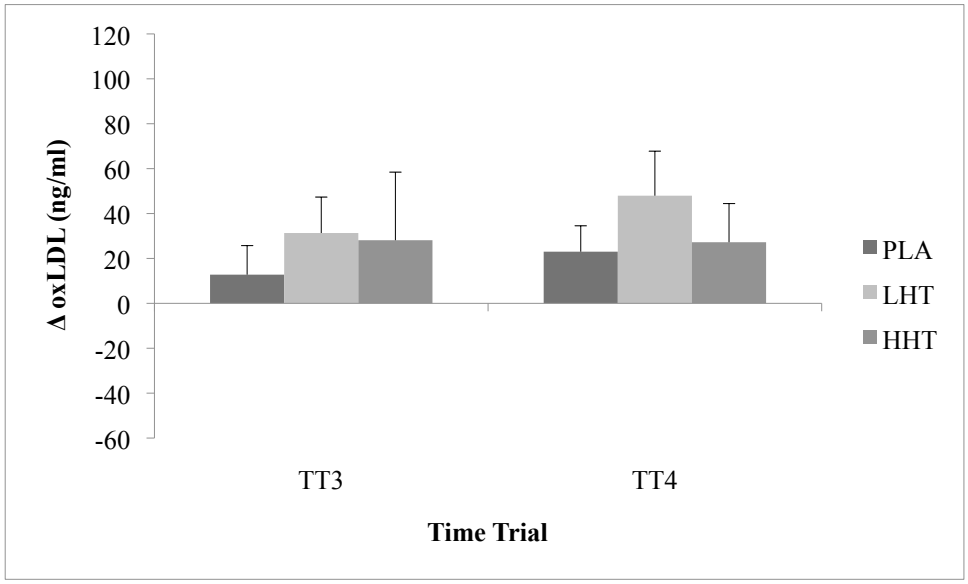

Figure 2.3. *Changes in oxLDL (ng/ml) during exercise at TT3 and TT4 between treatments. Bars represent means ± S.E. No significant acute treatment effect was found.*

CRP

There was no significant chronic treatment effect between TT1, TT2, and TT3 resting CRP values, but a significant treatment-by-time interaction was found (p=.041). A post hoc analysis, however, showed no specific differences between treatments. Upon further analysis, we found significance in the Δ between TT1 and TT3 resting CRP levels (p=.018) with the post hoc analysis showing HHT is significantly lower than LHT (p=.006). No significant chronic treatment effect or treatment-by-time interactions were found during exercise between TT1, TT2, and TT3. Also, no significant acute treatment effects were found between TT3 and TT4 at rest or during exercise.

Table 3. *Descriptive data by time point and treatment for CRP (mg/L).*

	PLA		**LHT**		**HHT**	
	PRE	END	PRE	END	PRE	END
TT1	1.13 ± 0.30	1.27 ± 0.36	0.95 ± 0.18	1.05 ± 0.20	1.81 ± 0.53	2.18 ± 0.66
TT2	1.03 ± 0.20	1.11 ± 0.21	1.54 ± 0.54	1.58 ± 0.53	1.17 ± 0.27	1.31 ± 0.32
TT3	1.45 ± 0.38	1.62 ± 0.42	1.50 ± 0.30	1.60 ± 0.31	0.98 ± 0.25	1.20 ± 0.35
TT4	2.07 ± 0.52	2.30 ± 0.58	1.72 ± 0.31	1.89 ± 0.35	1.40 ± 0.33	1.55 ± 0.38

Values are means ± SE.

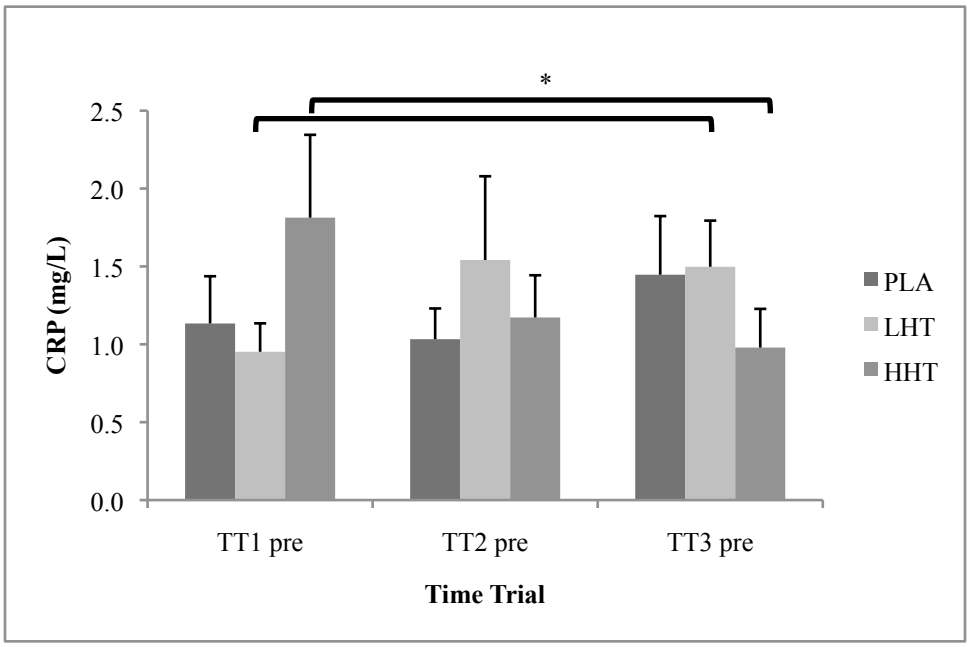

Figure 3.0. *Resting CRP levels (mg/L) at TT1, TT2, and TT3 between treatments. Bars represent means ± S.E. Brackets appear above time trials in which significance was found between Δ values. *Significantly lower than LHT (p=.006).*

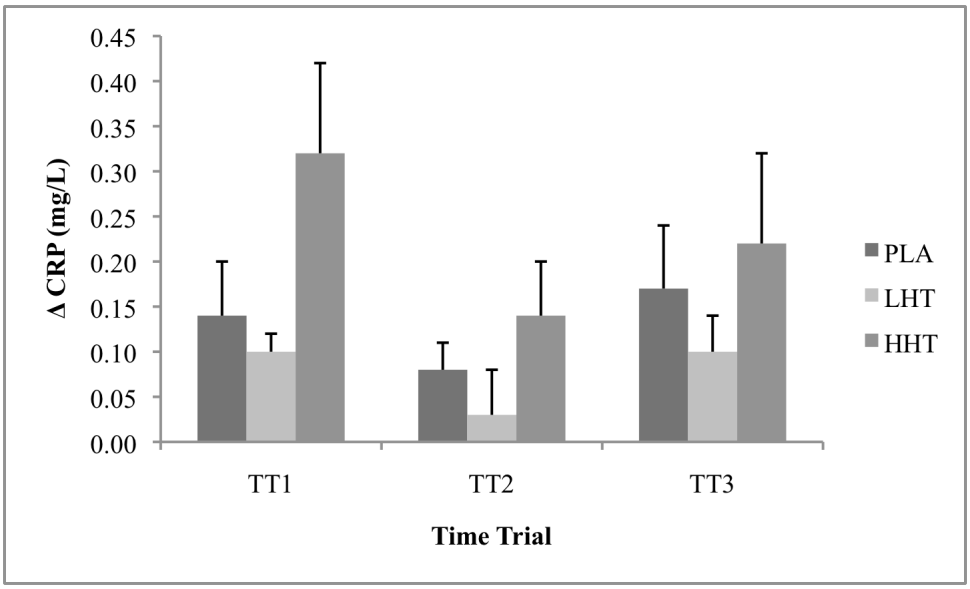

Figure 3.1. *Changes in CRP (mg/L) during exercise at TTI, TT2, and TT3 between treatments. Bars represent means ± S.E. No significant chronic treatment effect or treatment-by-time interaction was found.*

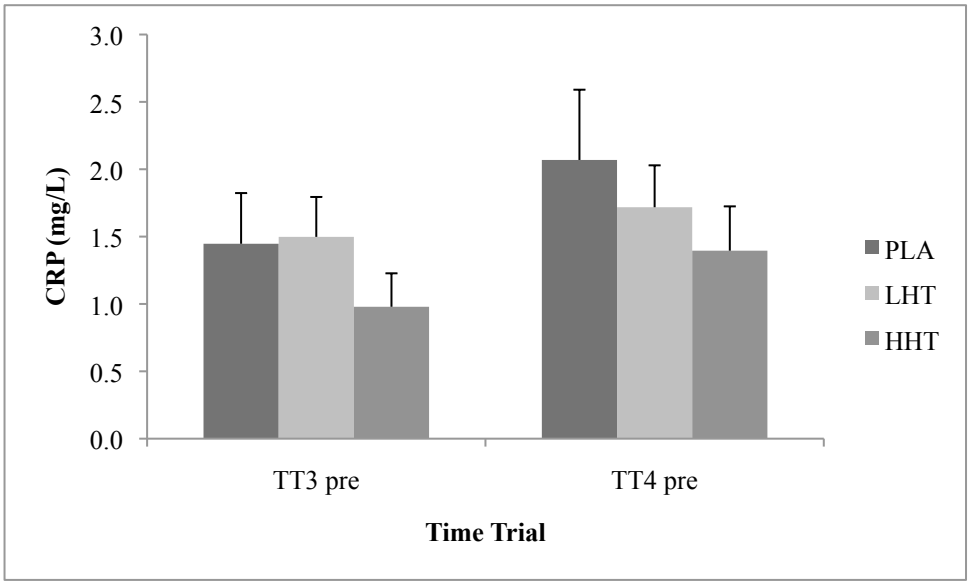

Figure 3.2. *Resting CRP levels (mg/L) at TT3 and TT4 between treatments. Bars represent means ± S.E. No significant acute treatment effect was found.*

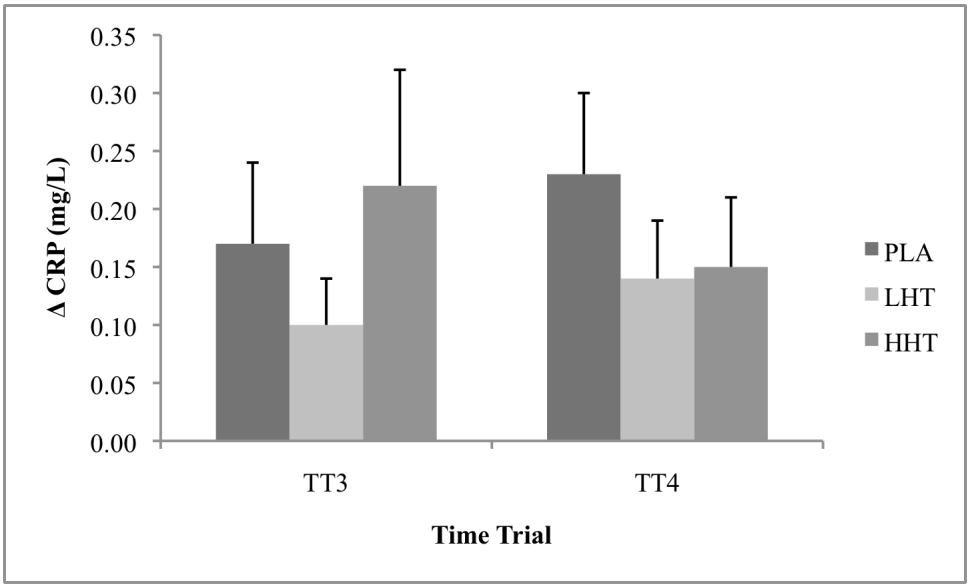

Figure 3.3. *Changes in CRP (mg/L) during exercise at TT3 and TT4 between treatments. Bars represent means ± S.E. No significant acute treatment effect was found.*

8IP

There was no significant chronic treatment effect between TT1, TT2, and TT3 resting 8IP values, but a significant treatment-by-time interaction was found (p=.003). The post hoc analysis, however, showed no specific differences between treatments. Upon further analysis, we found significance in the Δ between TT1 and TT2 resting 8IP levels (p=.001) with the post hoc analysis showing LHT is significantly higher than PLA (p=.001) and HHT (p=.005). We also found significance in the Δ between TT2 and TT3 resting 8IP levels (p=.012) with the post hoc analysis showing LHT is significantly lower than PLA (p=.003). There was a significant chronic treatment-by-time interaction between TT1, TT2, and TT3 during exercise (p=.031) with a post hoc analysis showing LHT significantly lower than PLA in TT2 (p=.027). No significant acute treatment effects were found between TT3 and TT4 at rest or during exercise.

Table 4. *Descriptive data by time point and treatment for 8IP (pg/ml).*

	PLA		**LHT**		**HHT**	
	PRE	END	PRE	END	PRE	END
TT1	35.30 ± 2.52	34.99 ± 3.33	31.93 ± 2.37	33.24 ± 2.26	33.54 ± 2.25	32.41 ± 2.22
TT2	31.53 ± 2.27	34.45 ± 2.51	37.16 ± 2.34	33.61 ± 3.04	31.86 ± 1.77	34.23 ± 1.90
TT3	32.88 ± 2.16	35.28 ± 2.96	31.66 ± 1.90	32.86 ± 1.99	30.23 ± 1.73	32.99 ± 2.02
TT4	30.37 ± 2.64	33.40 ± 2.65	30.08 ± 1.83	34.03 ± 2.16	31.65 ± 2.19	35.84 ± 2.48

Values are means ± SE.

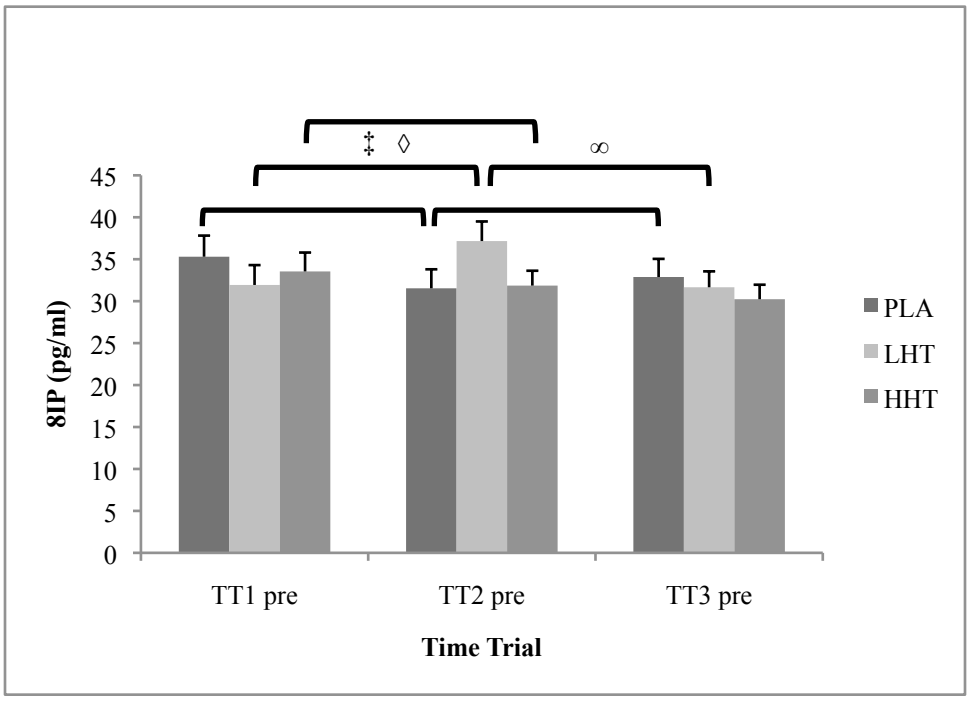

Figure 4.0. *Resting 8IP levels (pg/ml) at TT1, TT2, and TT3 between treatments. Bars represent means ± S.E. Brackets appear above time trials in which significance was found between Δ values. ‡Significantly higher than PLA (p=.001) between TT1 and TT2. ◊Significantly higher than HHT (p=.005) between TT1 and TT2. ∞Significantly lower than PLA (p=.003) between TT2 and TT3.*

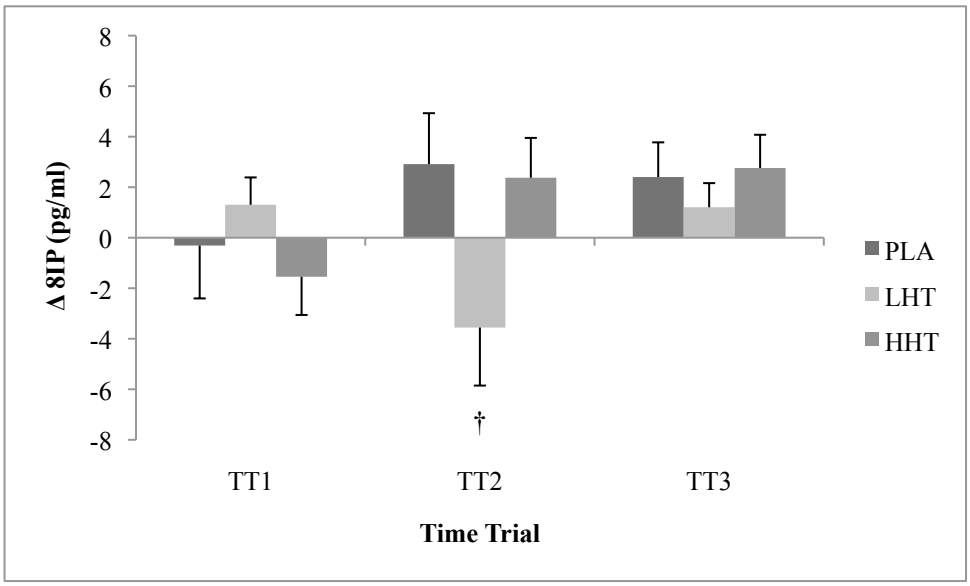

Figure 4.1. *Changes in 8IP (pg/ml) during exercise at TTI, TT2, and TT3 between treatments. Bars represent means ± S.E. †Significantly lower than PLA (p=.027) in TT2.*

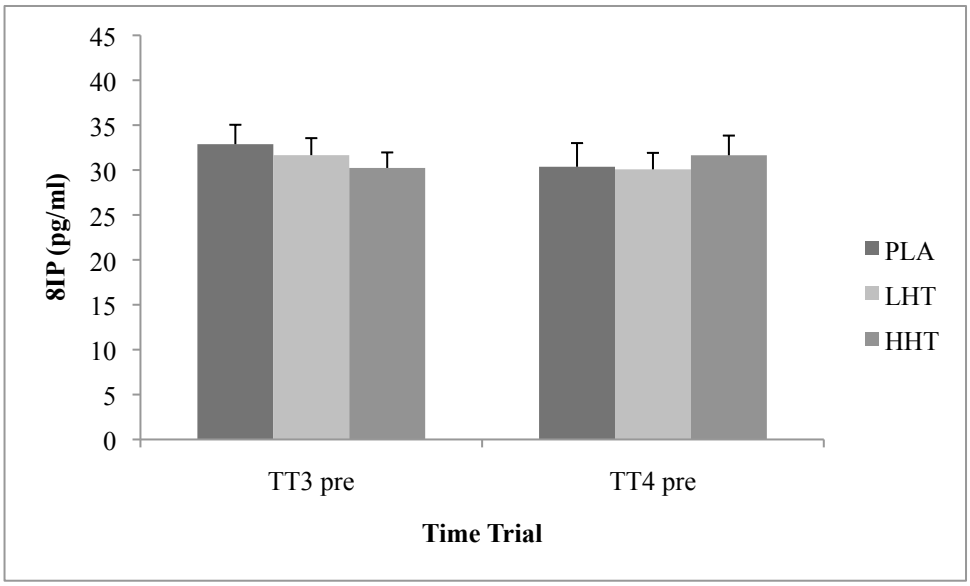

Figure 4.2. *Resting 8IP levels (pg/ml) at TT3 and TT4 between treatments. Bars represent means ± S.E. No significant acute treatment effect was found.*

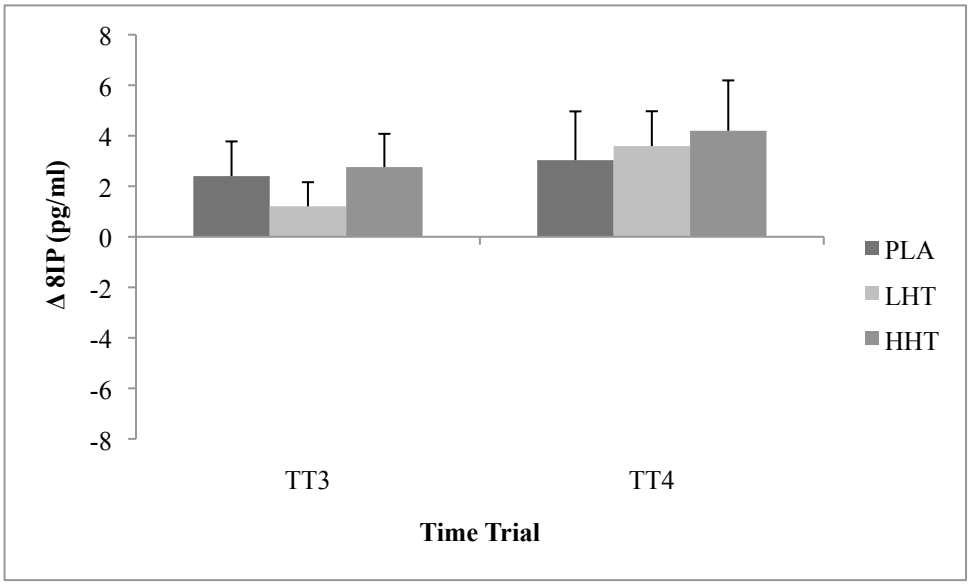

Figure 4.3. *Changes in 8IP (pg/ml) during exercise at TT3 and TT4 between treatments. Bars represent means ± S.E. No significant acute treatment effect was found.*

TNFα

There was no significant chronic treatment effect or treatment-by-time interaction between TT1, TT2, and TT3 at rest or during exercise. A significant acute treatment effect was found in the Δ between TT3 and TT4 for resting TNFα values (p=.045) with the post hoc analysis showing LHT is significantly lower than HHT (p=.014). No significant acute treatment effect was found during exercise between TT3 and TT4.

Table 5. *Descriptive data by time point and treatment for TNFα (pg/ml).*

	PLA		LHT		HHT	
	PRE	END	PRE	END	PRE	END
TT1	5.59 ± 0.64	7.21 ± 1.26	6.69 ± 0.43	7.83 ± 0.54	5.93 ± 0.49	7.48 ± 0.62
TT2	7.57 ± 2.26	10.14 ± 2.92	6.03 ± 0.43	7.63 ±0.69	5.37 ± 0.53	6.85 ± 0.69
TT3	7.08 ± 1.37	8.61 ± 1.54	6.51 ± 0.61	7.58 ± 0.64	5.32 ± 0.59	6.67 ± 0.58
TT4	6.64 ± 1.07	8.66 ± 1.67	5.97 ± 0.47	7.31 ± 0.63	5.65 ± 0.57	6.71 ± 0.68

Values are means ± SE.

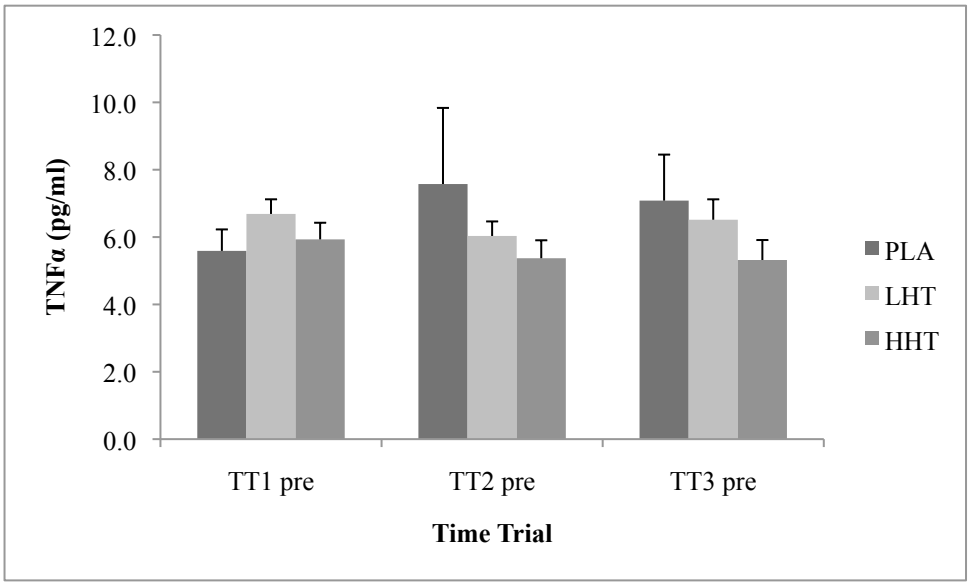

Figure 5.0. *Resting TNFα levels (pg/ml) at TT1, TT2, and TT3 between treatments. Bars represent means ± S.E. No significant chronic treatment effect or treatment-by-time interaction was found.*

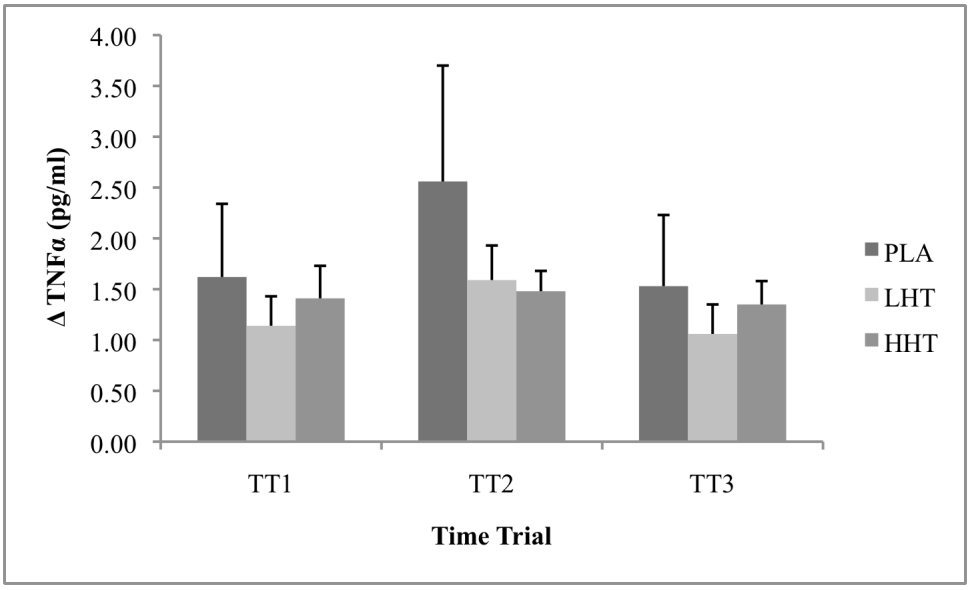

Figure 5.1. *Changes in TNFα (pg/ml) during exercise at TTI, TT2, and TT3 between treatments. Bars represent means ± S.E. No significant chronic treatment effect or treatment-by-time interaction was found.*

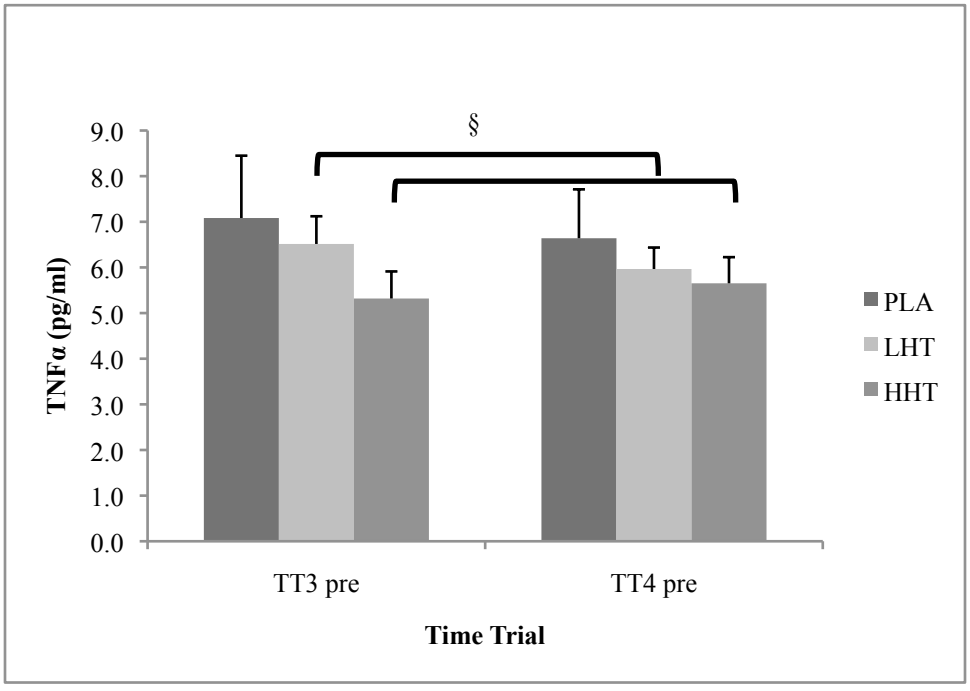

Figure 5.2. *Resting TNFα levels (pg/ml) at TT3 and TT4 between treatments. Bars represent means ± S.E. Brackets appear above time trials in which significance was found between Δ values. §Significantly lower than HHT (p=.014).*

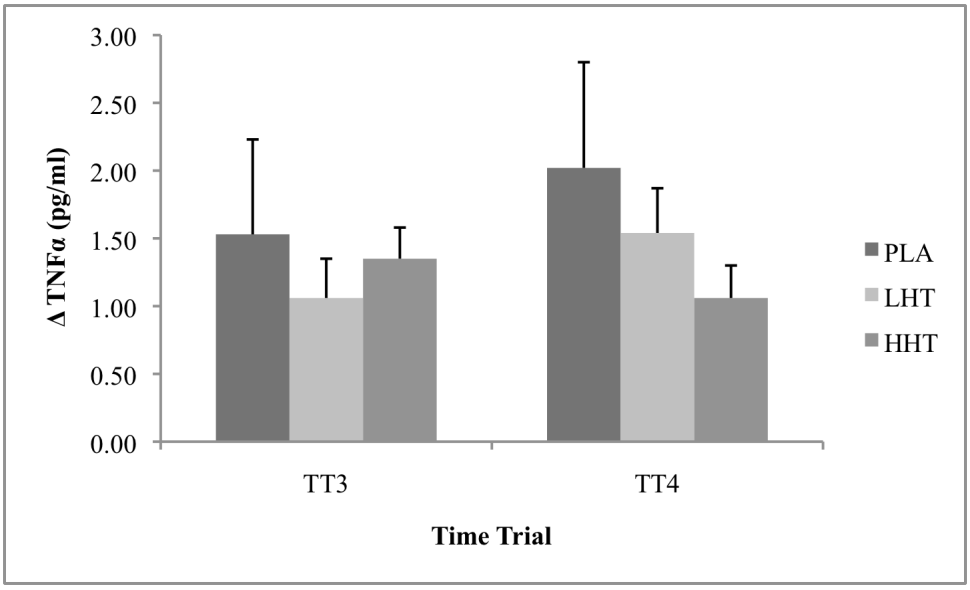

Figure 5.3. *Changes in TNFα (pg/ml) during exercise at TT3 and TT4 between treatments. Bars represent means ± S.E. No significant acute treatment effect was found.*

IL-6

There was no significant chronic treatment effect between TT1, TT2, and TT3 resting IL-6 values, but a significant treatment-by-time interaction was found (p=.033). A post hoc analysis, however, showed no specific differences between treatments. With further analysis, we found significance in the Δ between TT1 and TT3 resting IL-6 levels (p=.032) with the post hoc analysis showing HHT is significantly lower than PLA (p=.009). There was no significant chronic treatment-by-time interaction between TT1, TT2, and TT3 during exercise. A significant acute treatment effect was found in the Δ between TT3 and TT4 for resting IL-6 levels (p=.026) with the post hoc showing HHT is significantly higher than PLA (p=.009). No significant acute treatment effect was found during exercise between TT3 and TT4.

Table 6. *Descriptive data by time point and treatment for IL-6 (pg/ml).*

	PLA		LHT		HHT	
	PRE	END	PRE	END	PRE	END
TT1	1.43 ± 0.13	1.96 ± 0.13	1.50 ± 0.18	1.92 ± 0.15	1.74 ± 0.18	2.22 ± 0.14
TT2	1.71 ± 0.19	2.14 ± 0.19	1.49 ± 0.18	1.96 ± 0.14	1.65 ± 0.15	2.14 ± 0.12
TT3	1.72 ± 0.19	2.21 ± 0.17	1.55 ± 0.20	2.01 ± 0.16	1.63 ± 0.14	2.18 ± 0.11
TT4	1.62 ± 0.17	2.04 ± 0.16	1.50 ± 0.19	1.88 ± 0.18	1.72 ± 0.14	2.09 ± 0.12

Values are means ± SE.

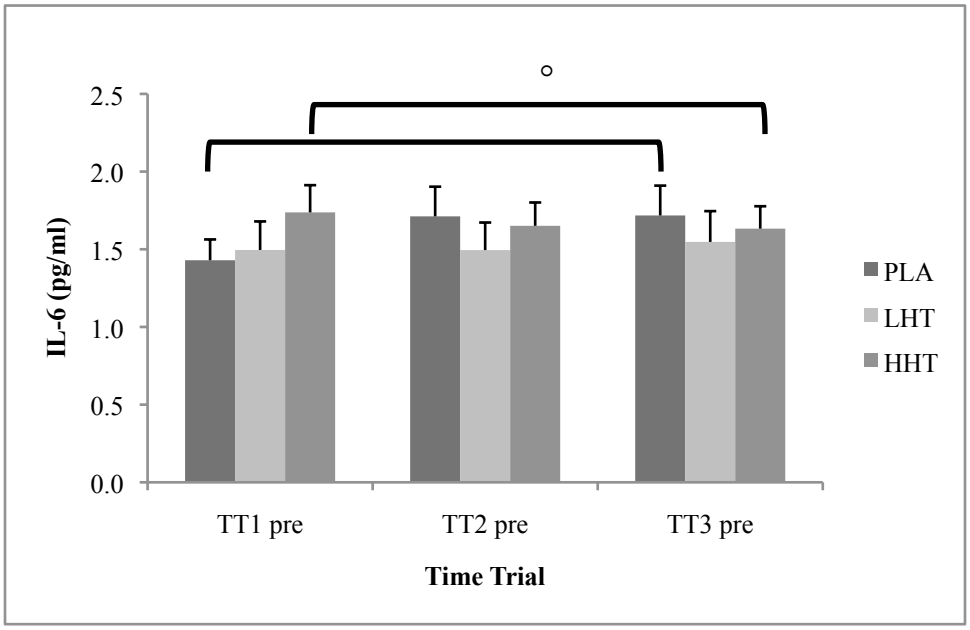

Figure 6.0. *Resting IL-6 levels (pg/ml) at TT1, TT2, and TT3 between treatments. Bars represent means ± S.E. Brackets appear above time trials in which significance was found between Δ values. °Significantly lower than PLA (p=.009).*

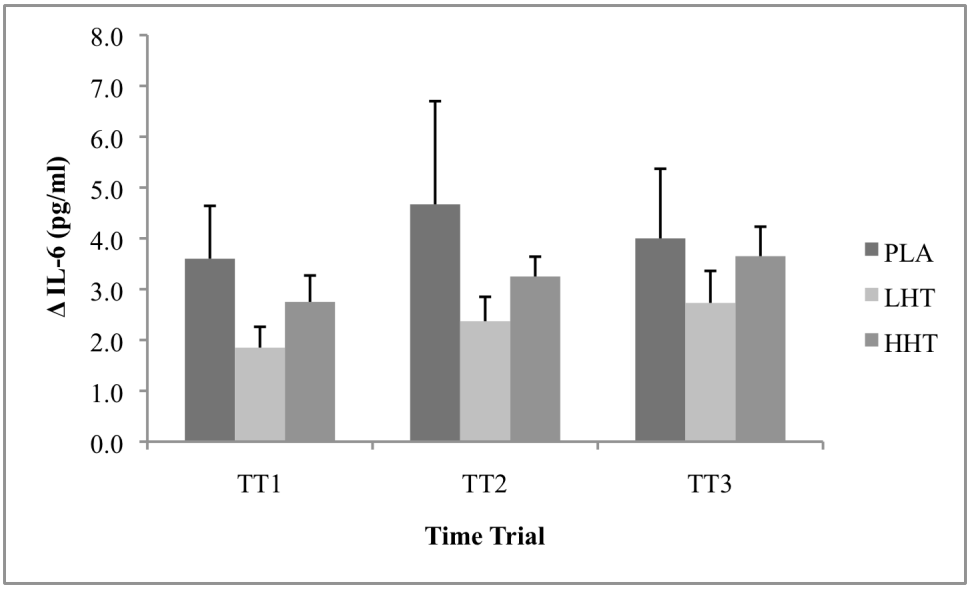

Figure 6.1. *Changes in IL-6 (pg/ml) during exercise at TTI, TT2, and TT3 between treatments. Bars represent means ± S.E. No significant chronic treatment effect or treatment-by-time interaction was found.*

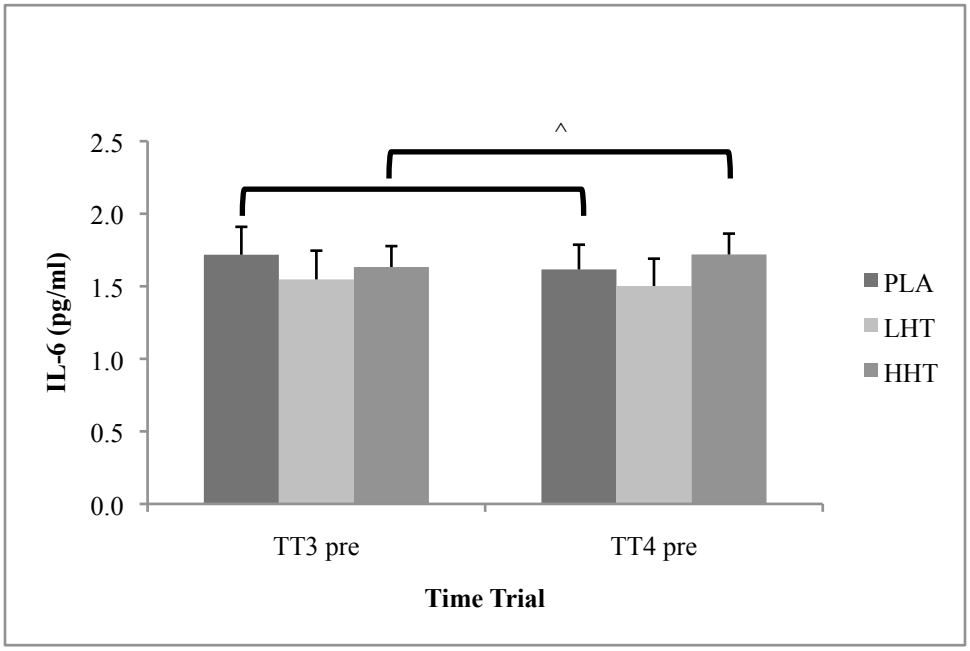

Figure 6.2. *Resting IL-6 levels (pg/ml) at TT3 and TT4 between treatments. Bars represent means ± S.E. Brackets appear above time trials in which significance was found between Δ values. ^Significantly higher than PLA (p=.026).*

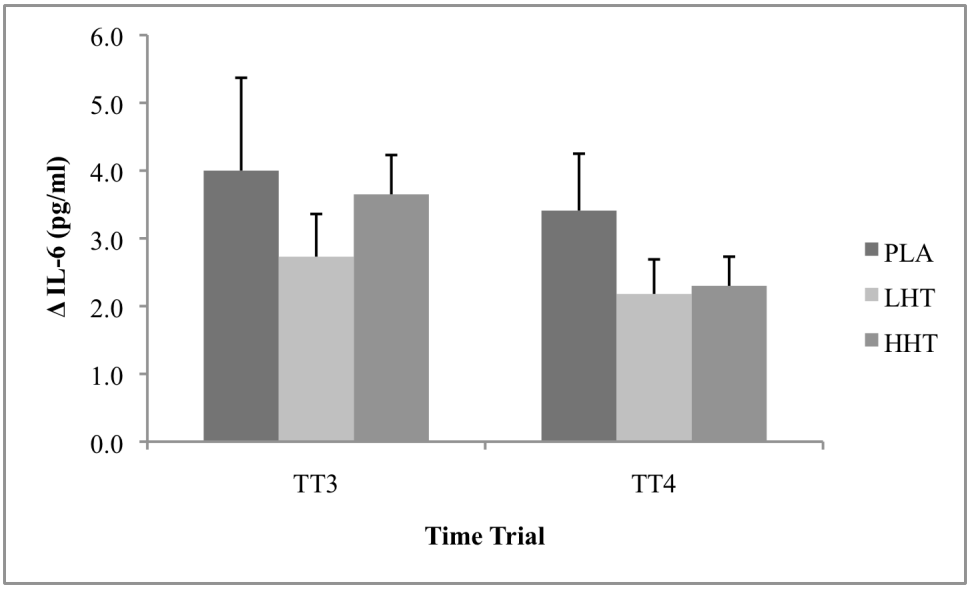

Figure 6.3. *Changes in IL-6 (pg/ml) during exercise at TT3 and TT4 between treatments. Bars represent means ± S.E. No significant acute treatment effect was found.*

IL-10

There was no significant chronic treatment effect or treatment-by-time interaction between TT1, TT2, and TT3 at rest or during exercise. Also, no significant acute treatment effects were found between TT3 and TT4 at rest or during exercise.

Table 7. *Descriptive data by time point and treatment for IL-10 (pg/ml).*

	PLA		LHT		HHT	
	PRE	END	PRE	END	PRE	END
TT1	27.75 ± 12.04	36.03 ± 14.38	34.86 ± 17.06	37.06 ± 15.50	72.99 ± 38.67	71.76 ± 30.53
TT2	42.60 ± 18.79	76.25 ± 45.02	31.45 ± 17.65	37.05 ± 19.99	33.72 ± 13.57	42.20 ± 15.37
TT3	33.61 ± 9.51	50.46 ± 18.35	34.43 ± 14.96	42.61 ± 17.41	32.22 ± 10.80	42.63 ± 15.52
TT4	33.46 ± 10.14	41.89 ± 13.82	33.73 ± 18.23	37.54 ± 19.16	38.91 ± 14.07	40.95 ± 12.12

Values are means ± SE.

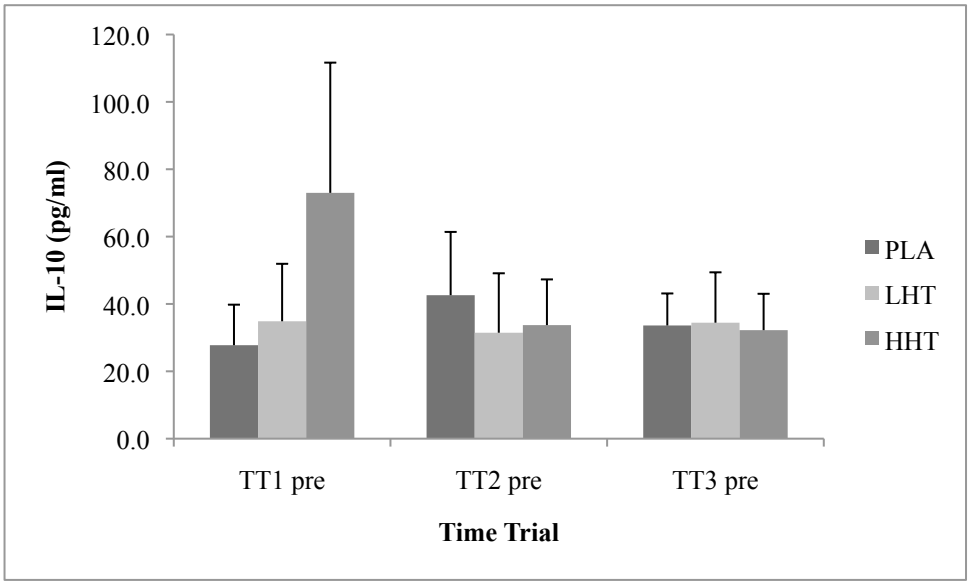

Figure 7.0. *Resting IL-10 levels (pg/ml) at TT1, TT2, and TT3 between treatments. Bars represent means ± S.E. No significant chronic treatment effect or treatment-by-time interaction was found.*

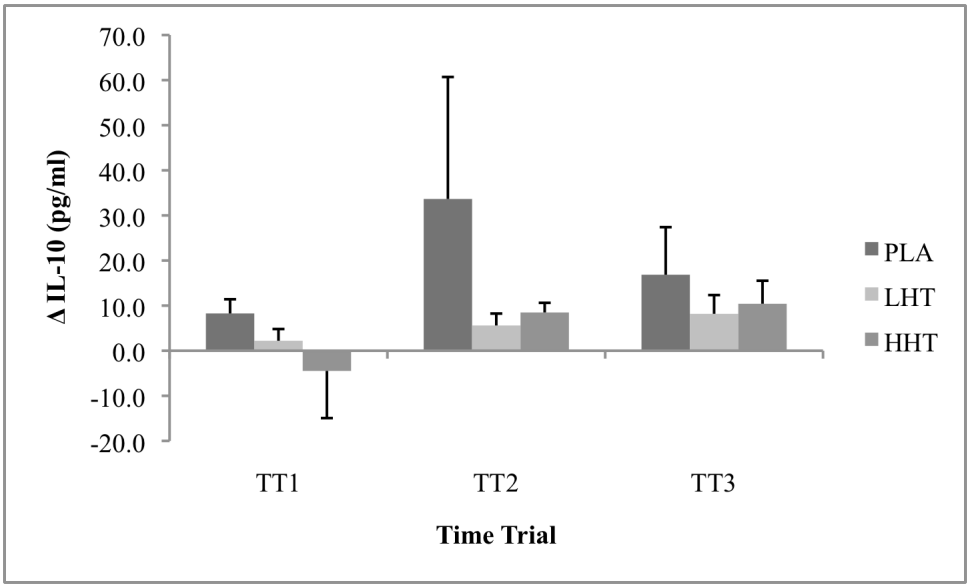

Figure 7.1. *Changes in IL-10 (pg/ml) during exercise at TTI, TT2, and TT3 between treatments. Bars represent means ± S.E. No significant chronic treatment effect or treatment-by-time interaction was found.*

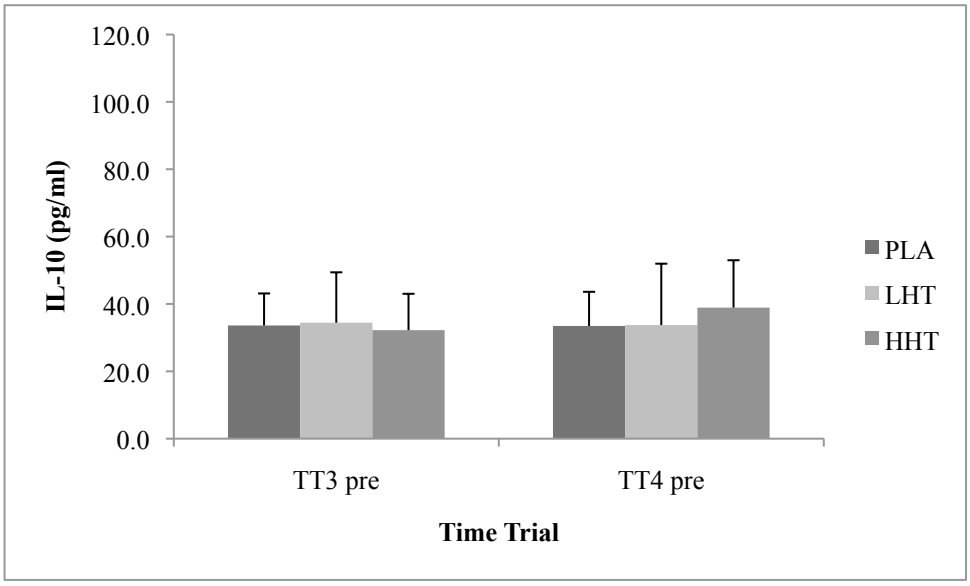

Figure 7.2. *Resting IL-10 levels (pg/ml) at TT3 and TT4 between treatments. Bars represent means ± S.E. No significant acute treatment effect was found.*

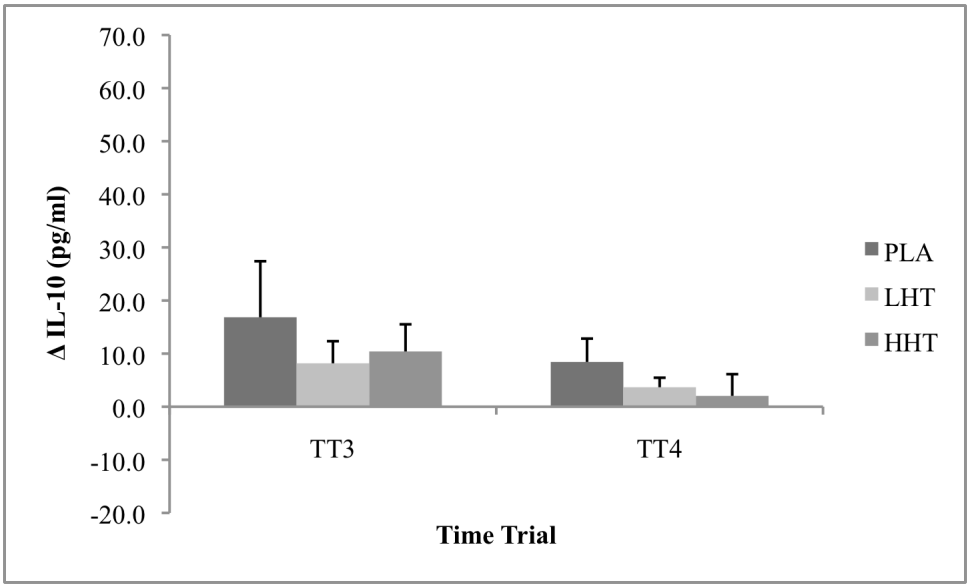

Figure 7.3. *Changes in IL-10 (pg/ml) during exercise at TT3 and TT4 between treatments. Bars represent means ± S.E. No significant acute treatment effect was found.*

IL-1β

There was no significant chronic treatment effect or treatment-by-time interaction between TT1, TT2, and TT3 at rest or during exercise. Also, no significant acute treatment effects were found between TT3 and TT4 at rest or during exercise.

Table 8. *Descriptive data by time point and treatment for IL-1β (pg/ml).*

	PLA		LHT		HHT	
	PRE	END	PRE	END	PRE	END
TT1	0.40 ± 0.18	0.51 ± 0.19	0.23 ± 0.09	0.22 ± 0.09	0.55 ± 0.23	0.80 ± 0.30
TT2	2.39 ± 1.79	2.69 ± 1.98	0.33 ± 0.14	0.39 ± 0.17	0.40 ± 0.15	0.59 ± 0.27
TT3	1.55 ± 0.80	1.76 ± 1.01	0.54 ± 0.28	0.49 ± 0.22	0.66 ± 0.27	0.65 ± 0.26
TT4	1.30 ± 0.76	1.86 ± 1.10	0.32 ± 0.16	0.59 ± 0.26	0.61 ± 0.24	0.64 ± 0.24

Values are means ± SE.

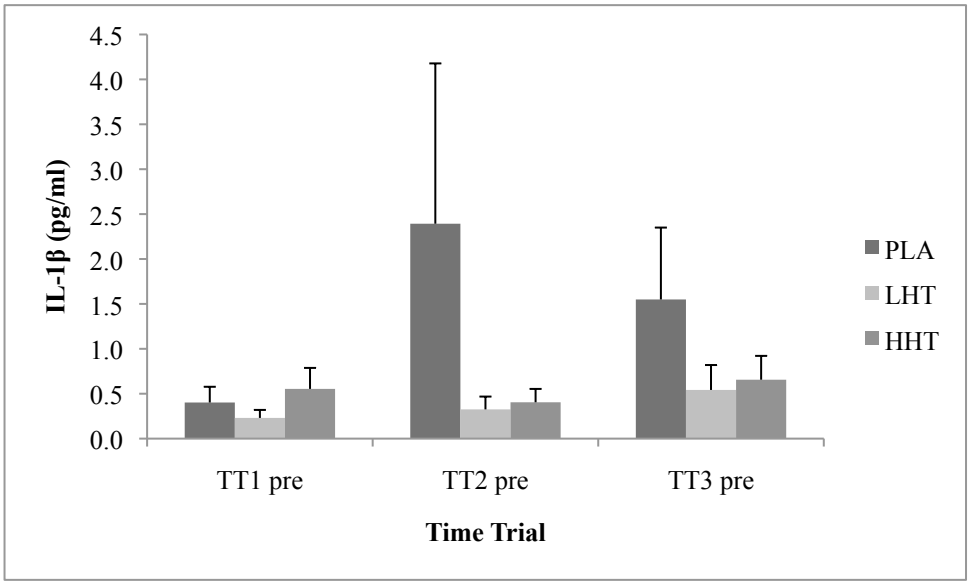

Figure 8.0. *Resting IL-1β levels (pg/ml) at TT1, TT2, and TT3 between treatments. Bars represent means ± S.E. No significant chronic treatment effect or treatment-by-time interaction was found.*

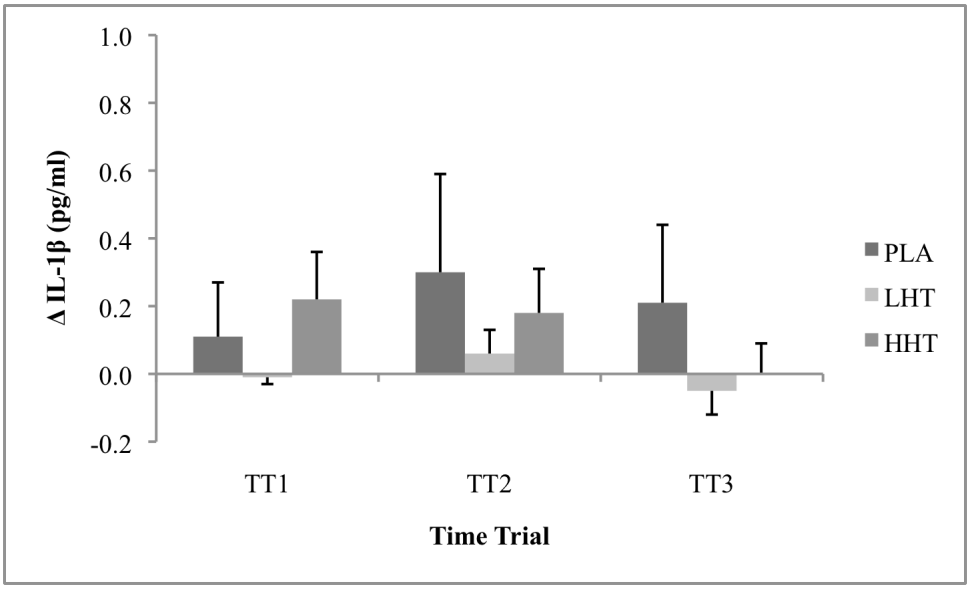

Figure 8.1. *Changes in IL-1β (pg/ml) during exercise at TTI, TT2, and TT3 between treatments. Bars represent means ± S.E. No significant chronic treatment effect or treatment-by-time interaction was found.*

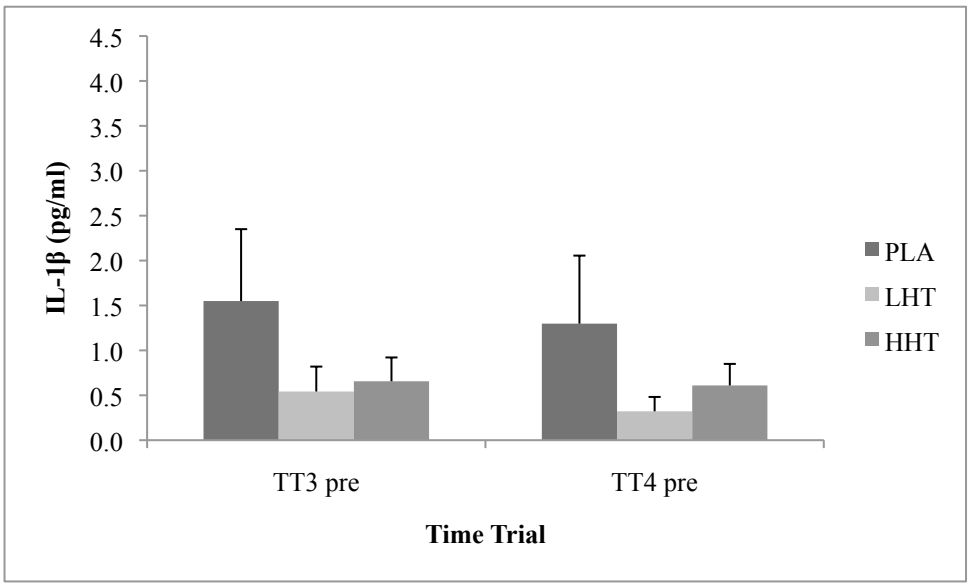

Figure 8.2. *Resting IL-1β levels (pg/ml) at TT3 and TT4 between treatments. Bars represent means ± S.E. No significant acute treatment effect was found.*

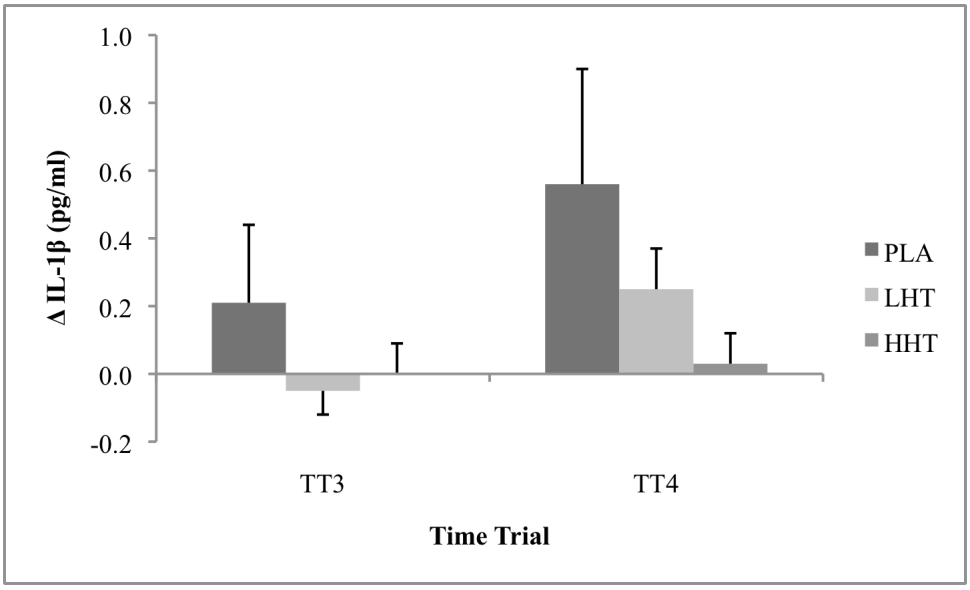

Figure 8.3. *Changes in IL-1β (pg/ml) during exercise at TT3 and TT4 between treatments. Bars represent means ± S.E. No significant acute treatment effect was found.*

IL-1ra

There was no significant chronic treatment effect or treatment-by-time interaction between TT1, TT2, and TT3 at rest or during exercise. Also, no significant acute treatment effects were found between TT3 and TT4 at rest or during exercise.

Table 9. *Descriptive data by time point and treatment for IL-1ra (pg/ml).*

	PLA		**LHT**		**HHT**	
	PRE	END	PRE	END	PRE	END
TT1	26.01 ± 10.29	27.41 ± 9.47	10.32 ± 3.66	13.74 ± 4.46	16.60 ± 7.11	20.58 ± 8.36
TT2	31.47 ± 11.36	40.14 ± 12.98	10.16 ± 3.24	11.24 ± 3.29	17.96 ± 6.13	21.12 ± 6.83
TT3	31.00 ± 11.42	35.33 ± 11.71	11.23 ± 3.72	13.50 ± 3.44	18.25 ± 7.57	22.44 ± 7.60
TT4	30.84 ± 10.51	34.04 ± 10.10	10.29 ±3.46	10.96 ± 3.34	19.57 ±7.97	21.44 ± 6.80

Values are means ± SE.

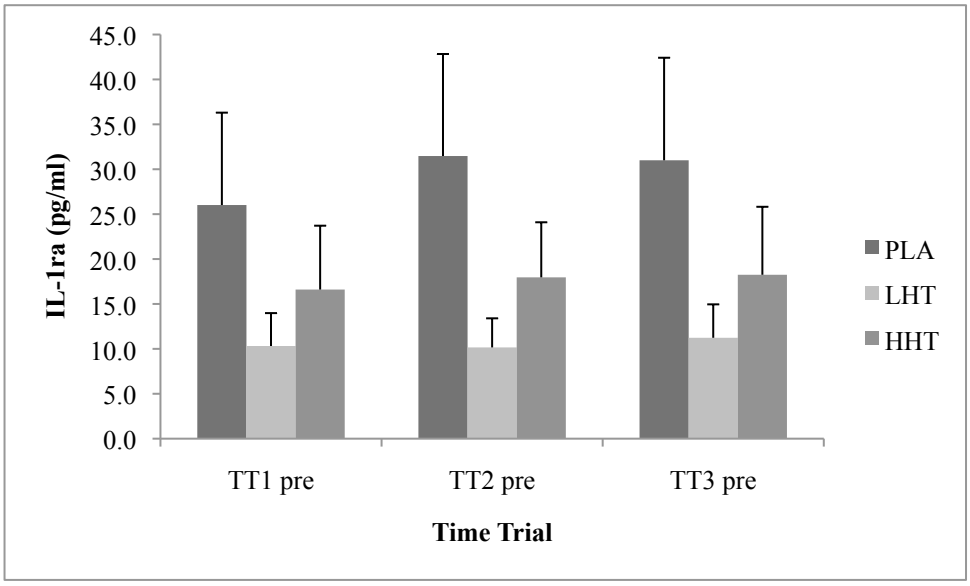

Figure 9.0. *Resting IL-1ra levels (pg/ml) at TT1, TT2, and TT3 between treatments. Bars represent means ± S.E. No significant chronic treatment effect or treatment-by-time interaction was found.*

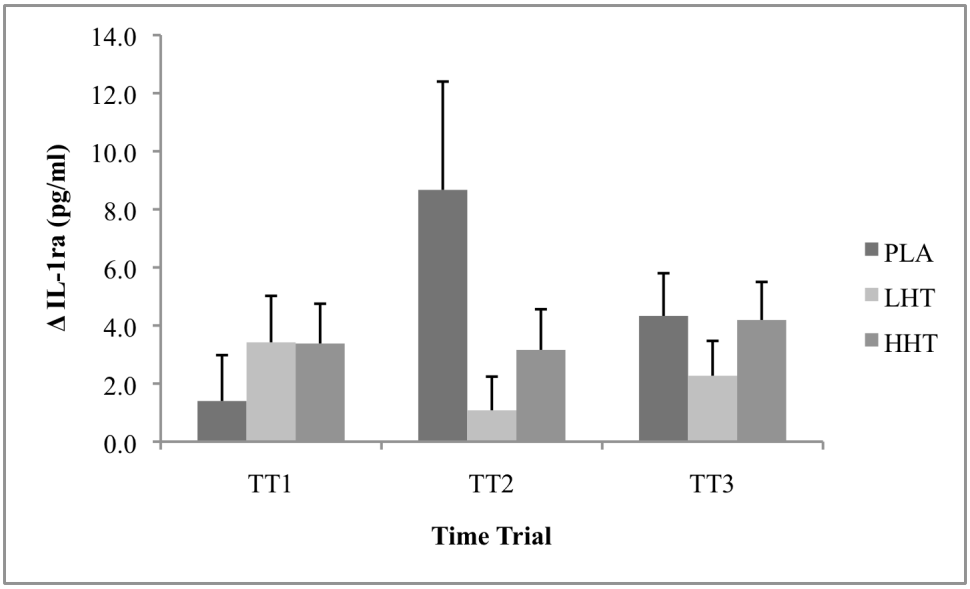

Figure 9.1. *Changes in IL-1ra (pg/ml) during exercise at TTI, TT2, and TT3 between treatments. Bars represent means ± S.E. No significant chronic treatment effect or treatment-by-time interaction was found.*

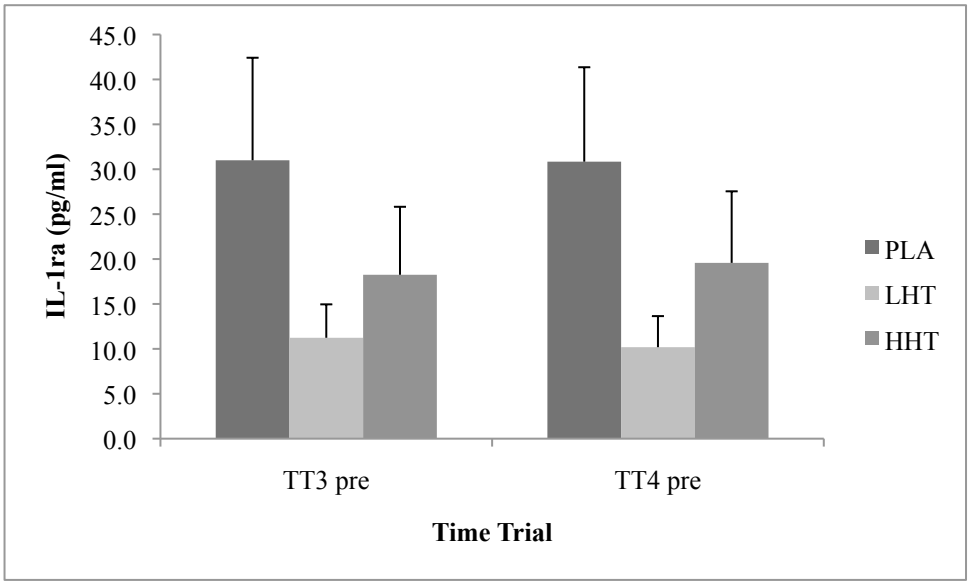

Figure 9.2. *Resting IL-1ra levels (pg/ml) at TT3 and TT4 between treatments. Bars represent means ± S.E. No significant acute treatment effect was found.*

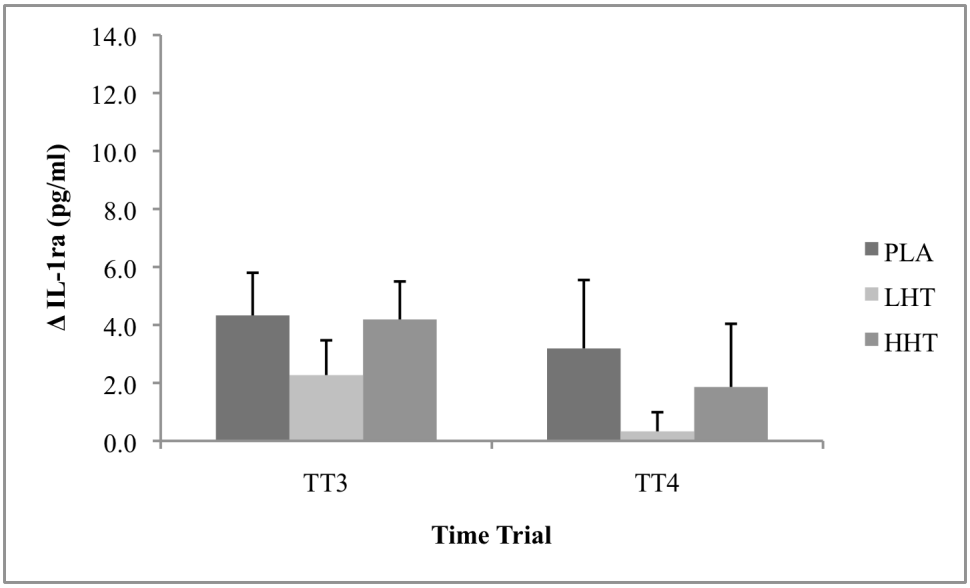

Figure 9.3. *Changes in IL-1ra (pg/ml) during exercise at TT3 and TT4 between treatments. Bars represent means ± S.E. No significant acute treatment effect was found.*

DISCUSSION

It is widely accepted that a Mediterranean diet contributes to a decreased disease risk and has an effect on overall health (Bogani et al., 2007; Carluccio et al., 2003; Cicerale et al., 2010; Covas, 2007; Minich & Bland, 2008; Pellegrini et al., 2003; Psaltopoulou et al., 2004). Olive oil is the primary source of fat in the Mediterranean diet and contains a considerable amount of phenols, which primarily contribute to decreasing oxidative stress and inflammation associated with many disease states (Cicerale et al., 2010; Covas, 2007; McDonald et al., 2001). In addition to a healthy diet, physical activity is also related to a decreased risk of cardiovascular morbidity and mortality (Mataix et al., 2008). Although exercise is beneficial, sufficient intensity or duration of an acute bout can induce oxidative stress (Teixeira et al., 2009). This occurs because a high demand is placed on skeletal muscle mitochondria to produce energy for muscle contraction and movement. The mitochondria become overpowered and release highly reactive oxygen species, which increase the inflammatory response (Mataix et al., 1998).

Therefore, the purpose of this study was to investigate the chronic and acute effects of a daily low dose (LHT, 50 mg/day) or daily high dose (HHT, 150 mg/day) of HT, one of the highest concentrated phenols found in olive oil, for 6 weeks over placebo conditions at rest and during exercise on total antioxidant status and markers of inflammation in healthy, recreationally active males. To the best of our knowledge, no study-to-date has examined the effects of HT supplementation at rest or during exercise over a 6-week period on antioxidant status and markers of inflammation in this population. Our primary findings indicate that there was no significant treatment effect

on markers of total antioxidant status – TEAC – and inflammation – oxLDL, CRP, 8IP, and cytokines – at rest or during exercise with 6 weeks of LHT or HHT. We did, however, observe significant treatment-by-time interactions for resting values of CRP, 8IP, and IL-6 over the 6-week period although no significant treatment differences were detected.

The change between TT1 and TT3 we observed in resting levels of CRP over the 6 week period was significantly lower for the HHT group compared to the LHT group, which actually increased over the course of the study. PLA also increased, but this was not significant. No significant differences, however, were observed with acute HT supplementation between TT3 and TT4 resting values. While our data shows a chronic decrease with a high dose of HT, this is similar to the significant decrease observed in patients with stable coronary heart disease (CHD) after consuming 50 ml of virgin olive oil daily for 3 weeks (Fito et al., 2008), which would equate to approximately 1.37 mg HT (Miro-Casas et al., 2003). We would expect the same decrease in CRP from TT1 to TT3 with LHT as this dose is well above the amount given in the aforementioned study; however, this was not the case. Compared to the patients with stable CHD, our sample included healthy, recreationally active males who engaged in weekly exercise but were not trained athletes, thus we would not expect elevated resting levels of CRP as epidemiological studies have reported the odds ratio for elevated CRP levels decreases with increased physical activity (Ford, 2002). Mean resting CRP levels throughout our study were well within normal limits of 0.068 to 8.2 mg/L (Tietz, 1995), and no significant elevations were observed during exercise between groups. We did see

elevated resting CRP levels at TT4, which occurred 24 hours after TT3, suggesting a persistent inflammatory effect of the exercise. Although the increased CRP levels were not significant, this agrees with Liesen et al. (1977) in which they detected a significant increase in CRP levels 1 and 2 days after prolonged physical exercise in young, healthy males.

CRP levels typically correlate with IL-6 levels (Du Clos & Mold, 2004; Vincent & Taylor, 2006) and thus, we observed similarly low, normal, resting IL-6 values throughout the study period. Although we did not observe significant differences between groups with the treatment-by-time interaction, the change in resting IL-6 values over the 6 week period was significantly lower for the HHT group compared to the PLA group, thus HHT decreased from TT1 to TT3 compared to an increase in PLA. The opposite effect, however, was seen in acute changes in resting values between TT3 and TT4. HHT increased compared to a decrease in PLA from TT3 to TT4. We also observed a significant increase in resting TNFα values from TT3 to TT4 in HHT compared to a decrease in LHT, and no significant chronic effect from TT1 to TT3 was observed for TNFα values at rest. Whether the significance found is a result of HT is yet to be determined as values were still well within the normal range (Himmerich et al., 2006). A lack of significant chronic effects as well as acute effects were present for resting levels of IL-10, IL-1β, and IL-1ra. This would be expected in this population because cytokines are not produced unless provoked by inflammatory agents, microbial invasion, or injury (Cerami, 1992).

It is likely to conclude that HT has no effect on the cytokines we measured during exercise because no significant chronic nor acute treatment effects were found between groups during time trials. Our findings support that of Teixeira et al., (2009), who concluded that their antioxidant cocktail, consisting of vitamins and minerals taken daily for 4 weeks, did not offer protection against exercise-induced inflammation as measured by IL-6 in trained kayakers after a 1000 m simulated kayak race. On the other hand, Vassilakopoulos et al., (2003) observed significant decreases in pro-inflammatory cytokines – IL-1β, IL-6, and TNFα – in post-exercise measurements after daily consumption for one month of a supplement containing various antioxidants such as vitamins, allopurinol and N-acetylcysteine, in healthy, untrained males. With these studies, it is difficult to isolate the effects of the ingredients without ruling out a synergistic effect among them all.

While we did not test for significant increases above baseline during exercise, IL-6 did increase 43% or more during exercise in all time trials and all treatments. This finding is in line with what is reported in the literature regarding release and subsequent increase of IL-6 from the contracting muscles during aerobic exercise (Gleeson, 2007). IL-6 is released regardless of skeletal muscle tissue damage and is related to the intensity, duration, mass of muscle recruited, and endurance capacity. The literature suggests that IL-6 released during exercise exhibits anti-inflammatory properties by stimulating the release of anti-inflammatory cytokines, IL-10 and IL-1ra, as well as soluble TNFα receptors, a naturally occurring inhibitor of TNFα (Petersen & Pedersen, 2005). IL-6 regulates TNFα levels as demonstrated in IL-6-deficient knockout mice with increased

resting levels of TNFα who required multiple doses of recombinant IL-6 to attneuate high TNFα levels (Mizuhara et al., 1994; Petersen & Pedersen, 2005). While slight, we also observed increases, although not directly tested for significance, during exercise in TNFα, IL-1ra, and IL-10 (except for a small and insignificant decrease in the PLA group at TT1) which could imply the effects of IL-6 released during exercise.

Another marker of inflammation we examined for this study was oxLDL, and we did not observe any significant chronic or acute treatment effects for resting levels of oxLDL. The current literature shows a strong association between HT and decreased oxLDL both *in vitro* and *in vivo*. For example, an inverse dose-response relationship exists between higher concentrations of HT and lower levels of peroxyl radical destruction of the LDL particle *in vitro* (Aruoma et al., 1998). In studies on rabbits, the ability of LDL to resist Cu^{2+}-induced oxidation was significantly increased when fed a modified standard diet containing 10% virgin olive oil and 7 mg/kg of oleuropein, a polyphenol similar in structure to HT and comparable to the average daily biophenol intake of a human consuming a Mediterranean diet, daily for 6 weeks when compared to rabbits on the standard diet (Coni et al., 2000). Vazquez-Velasco et al. (2010) observed significantly lower oxLDL levels in healthy human subjects after 3 weeks of consuming 45-50 mg/day of HT added to 10-15 g/day of sunflower oil when compared to the control group consuming only sunflower oil. While our study included doses above typical daily ingestion of HT, we did not find a chronic nor acute significant decrease in resting oxLDL levels between treatments despite the current research. There was no observable significant chronic or acute treatment effect during exercise on oxLDL. The present

study does not agree with findings from Liu et al. (1999), who observed an increase in the susceptibility of LDL to oxidation after a marathon race. The literature suggests that lipid hydroperoxides, as measured by oxLDL, increase during exercise (Urso & Clarkson, 2003); although we did not assess the sole effects of exercise on oxLDL, the changes during exercise were positive (aside from a negative change during TT1 in LHT) suggesting oxidative stress did occur during the exercise protocol. Despite a lack of significant findings with oxLDL, another marker of lipid peroxidation, 8IP, yielded some favorable results.

Even though there were no significant differences between groups with the treatment-by-time interaction, the change in resting 8IP values over the 3 week period from TT1 to TT2 was significantly higher for LHT compared to PLA and HHT; however, the opposite occurred in changes from TT2 to TT3 in which LHT was significantly lower than PLA. Although this finding was significant, the increase and subsequent decrease of 8IP in LHT was still within normal ranges of resting 8IP values (Wang et al., 1995), so it is improbable that HT had any real effect on this inflammatory marker. The literature suggests an inverse dose-response relationship exists between the rate of excretion of an isoprostane, specifically 8-iso-PGF_{2a}, and amounts of phenolics, or HT, ingested (Cornwell & Ma, 2008; Covas, 2007; Tuck & Hayball, 2002; Visioli et al., 2000; Visioli et al., 2001). This was not the case for our study – no treatment effect nor dose-response was observed. Similarly, Leger et al., (2005) examined the acute effects of HT and did not see a significant variation in 8-iso-PGF_{2a} urinary exretion during four-days of HT supplementation, in which the dose administered is typical of a southern European diet.

The subjects in this study, however, were Type I diabetics whose basal 8-iso-PGF_{2a} levels were already elevated three-fold; therefore, the authors attributed the lack of a significant finding in isoprostane excretion to the elevated basal levels. Although our participants' 8IP levels were normal, we still did not observe any significant acute changes in resting 8IP levels between TT3 and TT4.

Unlike the increases in 8IP during exercise observed in the literature, we observed a significant decrease in 8IP levels during exercise with LHT in TT2; however, this decrease was very diminutive (approximately 3.5 pg/ml). Mastaloudis et al. (2001) observed a significant increase in F_2-isoprostane levels of trained endurance runners following strenuous exercise when compared to the sedentary control protocol, and Goto et al. (2007) also reported a significant increase in 8-isoprostane levels following high intensity exercise. The mild and moderate intensity exercise protocols used in the latter study yielded insignificant differences among 8IP levels during exercise, which correspond to our findings. Goto and colleagues concluded that their high-intensity protocol was enough to bring about oxidative stress as evidenced by 8IP, which presents a possibility that our exercise protocol was not intense enough to elicit such results with either chronic or acute supplementation.

With the lack of significant findings between treatments for the markers of inflammation, it is probable to conclude that TEAC values were unaffected by the study product. Antioxidant consumption or supplementation is expected to increase antioxidant status and consequently decrease markers of inflammation; however, we observed no significant chronic or acute treatment effects in TEAC at rest or during exercise. Our

data, therefore, does not support that of Gonzalez-Santiago et al. (2006). They found a significant increase in TEAC when rabbits' diets were supplemented daily with 4 mg of HT/kg body weight for one month compared to the control group. Thus, this presents a limitation of our study; the rabbits' diets were standardized, and our participants' diets were not even though they were instructed to mimic their diet in the 48 hours preceding each time trial visit. Very little change was observed in TEAC levels at rest as well as during exercise throughout the course of the study. Berzosa et al. (2011) found antioxidant status significantly higher post-exercise when compared to pre-exercise regardless of a maximal or submaximal exercise model. We did not assess significance of end-exercise values compared to pre-exercise values; however, the change during exercise did not always increase. Our TEAC values were corrected for uric acid, so it is likely that endogenous uric acid production increased just as much as TEAC during exercise yielding an insignificant change in TEAC between treatments. Our data, therefore, more resembles that of Morillas-Ruiz and colleagues (2006) although they used trained cyclists versus our recreationally active group. The authors did not observe an acute significant difference in TEAC values after trained cyclists performed an exercise bout at 70% VO_{2max} both with and without an antioxidant beverage.

In conclusion, no significant treatment effects were found on chronic or acute measures of antioxidant status and inflammation at rest or during exercise with 6 weeks of LHT or HHT supplementation compared to PLA. We did observe significant treatment-by-time interactions for resting values of CRP, 8IP, and IL-6 over the 6-week period although no significant differences were detected between treatments. Therefore,

it is probable to conclude that chronic and acute HT supplementation does not improve antioxidant status nor decrease markers of inflammation in this population at rest or during exercise. This could be attributable to an already robust endogenous antioxidant defense as well as literature that report physically active individuals have lower chronic circulating levels of inflammatory biomarkers (Gleeson, 2007). The case could also be made for the lack of a vigorous exercise protocol because inflammatory markers were not significantly increased during exercise. Although participants performed subsequent days of exercise at TT3 and TT4 to examine possible amplified inflammatory effects of the exercise, the data suggests that this was not the case. It is possible to argue that there could be accumulation of HT, which would make it difficult to differentiate the chronic from the acute observations since TT4 was conducted after 6 weeks of supplementation. Anecdotal evidence from our lab implies that HT remained in the system for up to 1 week after consuming the last dose. Further studies should elucidate the acute effects of the antioxidant supplement prior to long-term ingestion. Also, studies examining the effects of HT in sick and diseased populations in combination with exercise could prove to be beneficial in decreasing markers of inflammation both from a diet and physical activity perspective.

References

Aruoma, O. I., Deiana, M., Jenner, A., Halliwell, B., Kaur, H., Banni, S., et al. (1998). Effect of Hydroxytyrosol Found in Extra Virgin Olive Oil on Oxidative DNA Damage and on Low-Density Lipoprotein Oxidation. *Journal of Agricultural and Food Chemistry*, 5181-5187.

Berzosa, C., Cebrian, I., Fuentes-Broto, L., Gomez-Trullen, E., Piedrafita, E., Martınez-Balların, E., et al. (2011). Acute Exercise Increases Plasma Total Antioxidant Status and Antioxidant Enzyme Activities in Untrained Men. *Journal of Biomedicine and Biotechnology*, 1-7.

Bogani, P., Gallie, C., Villa, M., & Visioli, F. (2007). Postprandial anti-inflammatory and antioxidant effects of extra virgin olive oil. *Atheroslerosis* (190), 181-186.

Carluccio, M. A., Siculella, L., Ancora, M. A., Massaro, M., Scoditti, E., Storelli, C., et al. (2003). Olive Oil and Red Wine Antioxidant Polyphenols Inhibit Endothelial Activation. *Arteriosclerosis, Thrombosis, and Vascular Biology, Journal of the American Heart Association*, 622-629.

Cerami, A. (1992). Inflammatory Cytokines. *Clinical Immunology and Immunopathology*, S3-S10.

Cicerale, S., Lucas, L., & Keast, R. (2010). Biological Activities of Phenolic Compounds Present in Virgin Olive Oil. *International Journal of Molecular Sciences*, 458-479.

Coni, E., Di Benedetto, R., Di Pasquale, M., Masella, R., Modesti, D., Mattei, R., et al. (2000). Protective Effect of Oleuropein, an Olive Oil Biophenol, on Low Density Lipoprotein Oxidizability in Rabbits . *Lipids*, 45-54.

Cornwell, D. G., & Ma, J. (2008). Nutritional Benefit of Olive Oil: The Biological Effects of Hydroxytyrosol and Its Arylating Quinone Adducts. *Journal of Agriculture and Food Chemistry*, 8774–8786.

Covas, M.-I. (2007). Olive oil and the cardiovascular system. *Pharmacological Research*, 175–186.

Du Clos, T. W., & Mold, C. (2004). C-Reactive Protein. *Immunologic Research* (30/3), 261-277.

Fito, M., Cladellas, M., de la Torre, R., Marti, J., Munoz, D., Schroder, H., et al. (2008). Anti-inflammatory effect of virgin olive oil in stable coronary disease patients: a

randomized, crossover, controlled trial. *European Journal of Clinical Nutrition*, 570–574.

Ford, E. S. (2002). Does Exercise Reduce Inflammation? Physical Activity and C-Reactive Protein Among U.S. Adults. *Epidemiology*, 561-568.

Gleeson, M. (2007). Immune function in sport and exercise. *Journal of Applied Physiology*, 693–699.

Gonzalez-Santiago, M., Martin-Bautista, E., Carrero, J., Fanolla, J., Baro, L., Bartolome, M., et al. (2006). One-month administration of hydroxytyrosol, a phenolic antioxidant present in olive oil, to hyperlipemic rabbits improves blood lipid profile, antioxidant status and reduces atherosclerosis development. *Atherosclerosis* (188), 35-42.

Goto, C., Nishioka, K., Umemura, T., Jitsuiki, D., Sakagutchi, A., Kawamura, M., et al. (2007). Acute Moderate-Intensity Exercise Induces Vasodilation Through an Increase in Nitric Oxide Bioavailiability in Humans. *American Journal of Hypertension*, 825–830.

Himmerich, H., Fulda, S., Linseisen, J., Seiler, H., Wolfram, G., Himmerich, S., et al. (2006). TNF-a, soluble TNF receptor and interleukin-6 plasma levels in the general population. *Eur. Cytokine Netw.*, 196-201.

Leger, C., Carbonneau, M., Michel, F., Mas, E., Monnier, L., Cristol, J., et al. (2005). A thromboxane effect of a hydroxytyrosol-rich olive oil wastewater extract in patients with uncomplicated type I diabetes. *European Journal of Clinical Nutrition*, 727–730.

Liesen, H., Dufaux, B., & Hollmann, W. (1977). Modifications of Serum Glycoproteins the Days Following a Prolonged Physical Exercise and the Influence of Physical Training. *European Journal of Applied Physiology and Occupational Physiology*, 243-254.

Liu, J., Shen, W., Zhao, B., Wang, Y., Wertz, K., Weber, P., et al. (2009). Targeting mitochondrial biogenesis for preventing and treating insulin resistance in diabetes and obesity: Hope from natural mitochondrial nutrients. *Advanced Drug Delivery Reviews*, 1343–1352.

Liu, M.-L., Bergholm, R., Makimattila, S., Lahdenpera, S., Valkonen, M., Hilden, H., et al. (1999). A marathon run increases the susceptibility of LDL to oxidation in vitro and modifies plasma antioxidants. *Am J Physiol Endocrinol Metab*, E1083-E1091.

Mastaloudis, A., Leonard, S. W., & Traber, M. G. (2001). OXIDATIVE STRESS IN ATHLETES DURING EXTREME ENDURANCE EXERCISE. *Free Radical Biology & Medicine*, 911-922.

Mataix, J., Battino, M., Ramirez-Tortosa, M. C., Bertoli, E., & Quiles, J. L. (2008). Virgin olive oil: a key healthy component of the Mediterranean diet. *Mediterr J Nutr Metab*, 69-75.

Mataix, J., Quiles, J. L., Huertas, J. R., Battino, M., & Manas, M. (1998). Tissue specific interactions of esercise, dietary fatty acids, and vitamin E in lipid peroxidation. *Free Radical Biology & Medicine, 24* (4), 511-521.

McDonald, S., Prenzler, P. D., Antolovich, M., & Robards, K. (2001). Phenolic content and antioxidant activity of olive extracts. *Food Chemistry*, 73-84 .

Minich, D. M., & Bland, J. S. (2008). Dietary management of the metabolic syndrome beyond macronutrients. *Nutrition Reviews, 66* (8), 429-444.

Miro-Casas, E., Covas, M.-I., Fito, M., Farre-Albadalejo, M., Marrugat, J., & de la Torre, R. (2003). Tyrosol and hydroxytyrosol are absorbed from moderate and sustained doeses of virgin olive oil in humans. *European Journal of Clinical Nutrition* (57), 186-190.

Mizuhara, H., O'Neill, E., Seki, N., Ogawa, T., Kusunoki, C., Otsuka, K., et al. (1994). T Cell Activation-associated Hepatic Injury: Mediation by Tumor Necrosis Factors and Protection by Interleukin 6. *J. Exp. Med.*, 1529-1537.

Morillas-Ruiz, J., Villegas Garcia, J., Lopes, F., Vidal-Guevara, M., & Zafrilla, P. (2006). Effects of polyphenolic antioxidants on exercise-induced oxidative stress. *Clinical Nutrition* (25), 444-453.

Patrick, L., & Uzick, M. (2001). Cardiovascular Disease: C-Reactive Protein and the Inflammatory Disease Paradigm: HMG-CoA Reductase Inhibitors, alpha-Tocopherol, Red Yeast Rice, and Olive Oil Polyphenols. A Review of the Literature. *Alternative Medicine Review*, 248-271.

Pellegrini, N., Serafini, M., Colombi, B., Del Rio, D., Salvatore, S., Bianchi, M., et al. (2003). Total Antioxidant Capacity of Plant Foods, Beverages and Oils Consumed in Italy Assessed by Three Different In Vitro Assays. *The Journal of Nutrition*, 2812-2819.

Petersen, A. M., & Pedersen, B. K. (2005). The anti-inflammatory effect of exercise. *Journal of Applied Physiology*, 1154 –1162.

Psaltopoulou, T., Naska, A., Orfanos, P., Trichopoulos, D., Mountokalakis, T., & Trichopoulou, A. (2004). Olive oil, the Meditteranean diet, and arterial blood pressure: the Greek European Prospective Investigation into Cancer and Nutrition (EPIC) study. *The American Journal of Clinical Nutritioin* (80), 1012-1018.

Raederstorff, D. (2009). Antioxidant activity of olive polyphenols in humans: a review. *International Journal for Vitamin and Nutrition Research*, 1-15.

Sporer, B., & McKenzie, D. C. (2007). Reproducibility of a Laboratory Based 20-km Time Trial Evaluation in Competitive Cyclists Using the Velotron Pro Ergometer. *International Journal of Sports Medicine* (28), 940-944.

Teixeira, V. H., Valente, H. F., Casal, S. I., Marques, A. F., & Moreira, P. A. (2009). Antioxidants Do Not Prevent Postexercise Peroxidation and May Delay Muscle Recovery. *Medicine & Science in Sports & Exercise*, 1752-1760.

Tietz, N. (Ed.). (1995). "Clinical Guide to Laboratory Tests". *(19106), 3rd*. Philadelphia, PA: W.B. Saunders Company.

Tuck, K. L., & Hayball, P. J. (2002). Major phenolic compounds in olive oil: metabolism and health effects. *The Journal of Nutritional Biochemistry* (13), 636-644.

Urso, M. L., & Clarkson, P. M. (2003). Oxidative stress, exercise, and antioxidant supplementation. *Toxicology*, 41-54.

Vassilakopoulos, T., Karatza, M.-H., Katsaounou, P., Kollintza, A., Zakynthinos, S., & Roussos, C. (2003). Antioxidants attenuate the plasma cytokine response to exercise in humans. *J Appl Physiol*, 1025–1032.

Vazquez-Velasco, M., Diaz, L. E., Lucas, R., Gomez-Martinez, S., Bastida, S., Marcos, A., et al. (2010). Effects of hydroxytyrosol-enriched sunflower oil consumption on CVD risk factors. *British Journal of Nutrition*, 1-5.

Vincent, H., & Taylor, A. (2006). Biomarkers and potential mechanisms of obesity-induced oxidant stress in humans. *International Journal of Obesity*, 400–418.

Visioli, F., & Galli, C. (1998). Olive Oil Phenols and Their Potential Effects on Human Health. *Journal of Agricultural and Food Chemistry*, 4292−4296.

Visioli, F., Caruso, D., Galli, C., Viappiani, S., Galli, G., & Sala, A. (2000). Olive Oils Rich in Natural Catecholic Phenols Decrease Isoprostane Excretion in Humans. *Biochemical and Biophysical Research Communications*, 797–799.

Visioli, F., Poli, A., & Galli, C. (2001). Antioxidant and Other Biological Activities of Phenols from Olives and Olive Oil. *Medicinal Research Reviews*, 65-75.

Wang, Z., Ciabattoni, G., Créminon, C., Lawson, J., Fitzgerals, G. A., Patrono, C., et al. (1995). Immunological characterization of urinary 8-epi-prostaglandin F2 alpha excretion in man. *The Journal of Pharmocology*, 94-100.

LITERATURE REVIEW

Introduction

Cardiovascular disease is one of the leading causes of death in industrialized nations and the second leading cause of death worldwide (Patrick & Uzick, 2001). However, epidemiological studies show that persons who consume a Mediterranean diet have a lower risk of all-cause and cause-specific mortality suggesting that this diet has an effect on overall health (Bogani et al., 2007; Carluccio et al., 2003; Cicerale et al., 2010; Covas, 2007; Minich & Bland, 2008; Pellegrini et al., 2003; Psaltopoulou et al., 2004). Olive oil is the primary source of fat in the Mediterranean diet and is associated with lower incidences of coronary heart disease and other degenerative diseases (Covas, 2007; Pellegrini et al., 2003). Because of its considerable benefits, researchers have begun to investigate the properties of olive oil that can attribute to the decreased risk of disease. For example, Fernández-Jarne et al. (2002) demonstrated that persons in the upper quintile of daily olive oil consumption had an 82% relative reduction in risk for a first non-fatal myocardial infarction even after adjustment for dietary and non-dietary confounders. The antioxidants found in olive oil may be a primary contributor to decreased disease markers. For example, hypercholesterolemic subjects fed olive oil consisting of 400 ppm of phenols for breakfast versus olive oil containing only 80 ppm of phenols exhibited a lower thrombogenic state, which is related to development of arteriosclerosis, two-hours after the meal (Ruano et al., 2007).

In conjunction with a healthy diet, physical activity is also associated with a decreased risk of cardiovascular morbidity and mortality (Mataix et al., 2008). Despite the associated health benefits of exercise, sufficient intensity or duration of physical activity can generate reactive oxygen species, which can increase oxidative stress often associated with an inflammatory state (Teixeira et al., 2009). This literature review will further investigate the properties of olive oil, its main antioxidant, hydroxytyrosol (HT), and how consumption can contribute to decreased oxidative stress and thus a decreased disease risk. It will then describe oxidative stress induced by exercise and how antioxidant supplementation may alter various markers of antioxidant status and inflammation.

Properties of Olive Oil

CONTENTS

Shelf life of oils is determined by antioxidant content, fatty acid composition of the lipids, and storage temperature (Mateos et al., 2003). Olive oil has a high monounsaturated fatty acid (MUFA) content comprised of 56-84% oleic acid (Galli & Visioli, 1999). This fatty acid exhibits protective properties against free radical damage and contributes to a longer shelf life when compared to polyunsaturated fatty acids (PUFA), such as linoleic acids that are typically found in other seed oils (Owen et al., 2000).

In addition to the high MUFA concentration, olives are a particularly rich source of phenolic antioxidants (McDonald et al., 2001). A high phenolic content protects the integrity of the oil from oxidation, termed oxidative stability, caused by environmental

stressors, such as ultraviolet (UV) radiation and high ambient temperatures (Visioli et al., 2001; Tuck & Hayball, 2002). Of all the phenols contained in olive oil, the three in highest concentration are the glycoside oleuropein, tyrosol, and hydroxytyrosol (3,4-dihydroxyphenyl ethanol, HT) (Tuck & Hayball, 2002). Concentrations of oleuropein and HT, both available as pure compounds, can be as high as 800 mg/kg in extra virgin olive oil and 2 g/100 g d.w. in olives (Carluccio et al., 2003; Visioli et al., 2001). In addition, the type of phenol, whether it is lipophilic or hydrophilic, can further enhance protection from oxidation in virgin olive oil. For example, Baldioli and colleagues (1996) found a higher hydrophilic content that was strongly correlated with oxidative stability (r = 0.97) in 48 different virgin olive oils. The question remains, however, can the protection against free radicals provided by high MUFA and phenolic concentrations be transferred *in vivo*?

In a study conducted by Huertas et al. (1999), rats were fed a semi-synthetic and isoenergetic diet that varied depending on the type of fat and antioxidant for 6 and 12 months from weaning. All rats were given either 50 mg/kg/day of virgin olive oil, sunflower oil, or sunflower oil supplemented with an antioxidant, coenzyme Q_{10} (CoQ10). After 6 or 12 months of daily olive oil consumption, cardiac muscle mitochondria were analyzed for the ratio of PUFA to MUFA as well as hydroperoxide free-radical assault on that tissue. Regardless of age, the cardiac muscle mitochondrial membrane fatty acid profile reflected the type of oil consumed; there was an increase in mitochondrial levels of oleic acids and a decrease in mitochondrial linoleic acid levels when fed virgin olive oil. Conversely, there was an increase in linoleic acid levels and a

decrease in oleic acid levels within the mitochondrial membrane fatty acid profile of rats consuming sunflower oil. The production of hydroperoxides tripled at 12 months of age in the diet containing only sunflower oil when compared to the virgin olive oil group. When CoQ10 was added to the sunflower oil, the production of hydroperoxides was significantly less compared to the sunflower oil group. The researchers concluded that addition of CoQ10 as a dietary supplement along with enrichment of the cardiac muscle mitochondrial membrane makeup by MUFA consumption, significantly protected against hydroperoxide attack on the aged mitochondrial membrane. Considering that olive oil's high MUFA and phenolic concentration can exert effects *in vivo*, many researchers have investigated its possible effects on attenuating similar disease processes.

EFFECT ON DISEASE

High oxidative stress levels in humans have been linked to disease, specifically atherosclerosis and cancer (McDonald et al., 2001). Because of the lower incidences of coronary heart disease and other degenerative diseases associated with olive oil consumption, researchers have begun to examine its effects on specific markers of oxidative stress. Chronic consumption of olive oil phenolic compounds can alter markers of oxidative stress by creating a favorable adjustment in lipid composition, platelet and cellular function, and a reducing inflammation (Cicerale et al., 2010).

Resistance of LDL to oxidation, a marker of atherogenesis, was increased when oleuropein, an olive oil polyphenol, was added to the diet of rabbits. The rabbits were fed a modified standard diet containing 10% virgin olive oil and 7 mg/kg of oleuropein daily for 6 weeks. Compared to the control group consuming the standard diet, the added

virgin olive oil and oleuropein group exhibited increased ability of LDL to resist oxidation through a Cu^{2+}-stimulated LDL conjugated diene formation (Coni et al., 2000). In another case, 23 hypertensive patients in a double-blind, crossover study were fed a diet high in MUFA from extra virgin olive oil for six months and then crossed over to a diet high in PUFA from sunflower oil for the remaining six months. When compared to PUFA, blood pressure and required daily doses of anti-hypertensive medication were significantly lower after MUFA consumption (Ferrara et al., 2000).

Furthermore, the type of olive oil, virgin or refined, can also contribute to decreased markers of oxidative stress. Fito et al. (2005) observed lower levels of oxidized lipoproteins and lipid peroxides and an increase in glutathione-peroxidase activity in males with stable coronary heart disease who consumed virgin olive oil. Subjects in this study consumed 50 ml of virgin olive oil per day for three-weeks and then consumed an equal amount of refined olive oil per day in another three-week period.

Likewise, phytochemicals in olive oil and red wine inhibit endothelial adhesion molecule expression, a marker of atherosclerosis, thus partially explaining cardio-protective benefits from a Mediterranean diet (Carluccio et al., 2003). To demonstrate, rabbits fed an olive leaf extract versus rabbits fed a high lipid diet for six weeks exhibited a down-regulation of atherosclerotic and inflammatory markers (Wang et al., 2008).

When compared to refined olive oil and other seed oils, virgin olive oil has a significantly higher phenolic antioxidant content and its polyphenol content can range from 100 mg/kg to 1000 mg/kg (D'Angelo et al., 2001; Owen et al., 2000). This

literature review will now further investigate one of the main phenols in olive oil, hydroxytyrosol.

Hydroxytyrosol (HT)

PHYSICAL AND CHEMICAL PROPERTIES

HT is a lipo- and hydro-soluble compound found within olives, and it is one of the most active and powerful antioxidants (Carrasco-Pancorbo et al., 2005; Deiana et al., 1999; Feng et al., 2011; Gordon et al., 2001). HT can range from 85-169 mg/kg in olive oil, and the progressive hydrolysis of oleuropein yields accumulation of HT and elenolic acid upon storage (Petroni et al., 1995; Cornwell & Ma, 2008; Granados-Principal et al., 2010). The antioxidant capability of phenols depends on the hydrogen-donating ability and successive stabilization of free radical molecules. Free radicals are atoms or molecules with an unpaired electron in an open shell configuration. By attempting to pair the unpaired valance electron, these molecules cause damage by oxidizing surrounding structures, specifically carbohydrates, lipids, proteins, and nucleic acids (Halliwell et al., 1995). Quiles et al. (2002) have suggested that the number of hydroxyl groups and insertion of an ethylenic group between the phenyl ring and carboxylate group determines the efficacy of these phenolics. HT is similar in structure to tyrosol with one hydroxyl group and one ethylenic group, except HT contains an additional hydroxyl group in the *meta* position (Tuck & Hayball, 2002). Caffeic acid, a similar compound also found in olive oil, contains only two hydroxyl groups and no ethylenic groups. Due to the lower number of hydoxyl and ethylenic groups, neither tyrosol nor caffeic acid have been shown to be as effective as HT in their antioxidant capabilities.

Once absorbed and metabolized, antioxidants exert their effects *in vivo*. HT is absorbed in the small intestine in a dose-dependent manner and excreted in urine in concentrations ranging from 30-60% up to two-hours after ingestion along with its metabolites homovanillic acid and homovanillic alcohol (Fitó et al., 2007; Granados-Principal et al., 2010; Schwedhelm et al., 2003; Tuck & Hayball, 2002). Peak concentration of HT in plasma has been observed at 32 minutes after consuming approximately 25 ml of virgin olive oil; however, others have observed a peak concentration in plasma approximately one-hour after ingestion (Fitó et al., 2007; Miro-Casas et al., 2003). How much olive oil needs to be consumed to obtain an effective dose of HT? The concentration of HT found in olive oil is outlined below.

VOLUME FOUND IN OLIVE OIL

The volume of antioxidants found in olive oil is indicative of its quality and stability, and the concentration of antioxidants varies depending upon the level of processing, ripeness of olives at harvest, and climate (Petroni et al., 1995). Because virgin olive oil is less refined, it has the highest concentration of total raw phenolics with values ranging from 130-350 mg/kg and concentrations of hydroxytyrosol ranging from 2.6 – 27.0 mg/kg (Fitó et al., 2007; Quiles et al., 2002). With almost 80% of the phenolic compounds lost in the refining process, olive wastewater is also a potent source for phenolic compounds (Manna et al., 2005). Regardless of the source, researchers are using antioxidants derived from olive oil to better observe their effects on the various markers of oxidative stress.

ANTIOXIDANT CAPACITY AND EFFECT ON DISEASE

HT and its derivatives possess antioxidant, anti-thrombotic, anti-atherogenic, and anti-inflammatory properties, which contribute to attenuation of disease processes. They have the ability to decrease plasma levels of oxidized LDL and improve oxidative stress markers including lipid hydroperoxides, malondialdehyde, and activity of endogenous antioxidant defense systems such as glutathione peroxidase (Raederstorff, 2009; Liu et al., 2009).

Quiles et al. (2002) investigated the protective effects of HT, tyrosol, and caffeic acid on human prostate epithelium (PC3) because of this tissue's susceptibility to oxidative stress. The authors specifically measured oxidative damage to lipids and DNA when PC3 cells were incubated with hydrogen peroxide (H_2O_2). No cytotoxic effects at pharmacological concentrations were observed on the human prostate cells when first treated with varying concentrations of HT, tyrosol, or caffeic acid for 24 hours. When compared to control cells containing no phenolics and no H_2O_2 in the medium, a 12-fold increase in DNA damage was observed in cells treated with 60 μM of H_2O_2. However, a dose-response decrease in DNA damage to cells was observed with increasing amounts of HT (10 μM, 50 μM, 100 μM, or 250 μM). Compared to tyrosol and caffeic acid, cells incubated in HT exhibited significantly less DNA damage at the given doses. Lipid peroxidation in PC3 cells incubated in HT was significantly less than controls and those treated with tyrosol or caffeic acid, but no dose-response effects with HT were observed. Thus, HT exhibited protective effects against oxidation of PC3 cells *in vitro* but not always in a dose-dependent manner.

Furthermore, a study by Kies and colleagues (2007) reported a dose-response relationship in male rat diaphragm muscle tissue with greater force production and a greater percent of protection when incubated with increasing doses of HT (0 μM, 10 μM, 25 μM, 50 μM, 100 μM, and 300 μM) and exposure to 1 mM H_2O_2 *in vitro*. H_2O_2 decreases initial force in the absence of HT; thus increased protection is exhibited in the presence of increasing amounts of HT. Another study conducted by Manna et al. (1997) showed HT-induced protection of intestinal cells against oxidative damage. Micromolar concentrations of HT (250 μmol/L) completely protected caco-2 intestinal cells from ROS assault induced by 10 mmol/L of H_2O_2 or 10 U/L of xanthine oxidase *in vitro*. The authors of this study concluded that dietary consumption of olive oil polyphenols might reduce the risk of oxidative stress-related gastrointestinal diseases.

This has led other researchers to investigate the effects of HT on other oxidative stress-induced diseases, in particular atherosclerosis. Because HT diminishes the cytokine-induced up-regulation of vascular adhesion molecules in human endothelial cells in culture, it was suggested that HT might reduce atherogenesis *in vivo* (Quiles et al., 2002). Also a study conducted by Petroni and others (1995) showed that concentrations of HT ranging from 100-400 μM completely inhibited platelet aggregation when induced by adenosine diphosphate (ADP) or collagen *in vitro*. At a dose of 400 μM, HT prevented collagen-induced aggregation of platelets and inhibited production of thromboxane-B_2 by stimulated platelets.

Another free radical associated with atherogenesis is peroxynitrite, the product of excess nitric oxide (NO^*) reacting with the superoxide radical (O_2^{*-}). NO^* is a necessary

physiological molecule, however, excess production can lead to chronic inflammation and cardiovascular disease via peroxynitrite damage. Peroxynitrite provokes peroxidation of lipids, depletes antioxidant systems, and causes DNA damage. The beneficial effects of HT to scavenge peroxynitrite have been confirmed *in vitro*, however, this effect *in vivo* is yet to be achieved (Deiana et al., 1999).

Although HT has demonstrated beneficial effects in reducing inflammation by scavenging free radicals both *in vitro* and *in vivo*, some researchers have shown a pro-oxidant effect with chronic HT consumption. For example, Faine et al. (2006) found a pro-oxidant effect on cardiac tissue of male Wistar rats after consuming HT twice per week for one month. The rodents were divided into four groups: control, olive oil (7.5 ml/kg), isolated oleic acid (3.45 ml/kg), and isolated HT (7.5 mg/kg) consumption. The rodents were gavaged twice a week with the respective treatment for a period of 30 days, and then lipid profiles in the blood and cardiac tissue were analyzed. Olive oil consumption showed a significant difference in improving the lipid profile, elevating HDL cholesterol, and diminishing LDL cholesterol concentrations. Conversely, a low-dose of isolated HT elicited an undesirable pro-oxidant effect in cardiac tissue of the rat model by increasing lipid hydroperoxide concentration as well as the ratio of lipid hydroperoxide to superoxide dismutase, an endogenous free radical scavenger. Moreover, an increase in the development of atherosclerotic lesions, total cholesterol, and circulating monocytes were seen when apo-E deficient mice were given high quantities of HT, 10 mg/kg/day, for a period of 10 weeks. Acin et al. (2006) concluded that isolated phenolic-enriched products could be potentially harmful given the results of their study.

Exercise and Free Radical Formation

Considering that antioxidants such as HT are typically associated with increased health benefits, but too much may be detrimental, the question arises can aerobic exercise yield similar confounding results? Epidemiological studies over the years have linked physical activity with decreased risk of cardiovascular morbidity and mortality; however, a paradox exists (Mataix et al., 2008). Oxidative stress is present in many disease states, and it is also a consequence of aerobic exercise. Aerobic exercise places a high demand on skeletal muscle mitochondria to produce energy for muscle contraction and movement. The influx of electrons, associated with the high-oxygen demands for physical activity, can overpower the mitochondria. Highly reactive oxygen species are then released because the last step of oxidative phosphorylation cannot be properly utilized (Mataix et al., 1998). Electrons leak out between transfers around complex-I and complex-III on the electron transport chain and interact with surrounding oxygen forming the superoxide anion. Superoxide dismutase (SOD), one of several endogenous enzymatic antioxidants, reacts with the superoxide anion forming hydrogen peroxide, a less offensive ROS, and oxygen (Mataix et al., 2008). Endogenous antioxidant systems typically combat the released ROS; however, if the endogenous antioxidant system is inadequate compared to the presence of pro-oxidants, oxidative stress can occur and trigger an inflammatory response activated by the interaction of ROS and skeletal muscle tissue (Viguie et al., 1993; Manna et al., 1999; Powers et al., 2004). The inflammatory response occurs post-exercise because exercise-induced muscle damage signals neutrophils to remove cellular debris, such as damaged muscular proteins. Monocytes

and macrophages are also summoned to the area, where they produce more ROS and pro-inflammatory cytokines, such as interleukin-1 (IL-1), tumor necrosis factor-alpha (TNFα), and interleukin-8 (IL-8), leading to overproduction of pro-oxidants. This puts the system into an imbalance, thereby increasing oxidative stress and amplifying the inflammatory response even further (Morillas-Ruiz et al., 2006; Teixeira et al., 2009).

Previous research on the exercise-induced inflammatory response and subsequent state of oxidant imbalance led Alessio and colleagues (2000) to measure markers of oxidative stress with exhaustive aerobic exercise and non-aerobic isometric exercise. The purpose of their study was to determine the different exercise effects on lipid peroxidation, protein oxidation, and total antioxidant status. After each exercise bout, evidence of oxidative stress was present in the blood of the 12 healthy subjects. Protein carbonyls were significantly increased post-exercise in the exhaustive aerobic exercise model, whereas, in the non-aerobic isometric exercise model, lipid hydroperoxides were significantly increased post-exercise. Total antioxidant status was significantly increased post exhaustive aerobic exercise versus pre-exercise values, but this was not significantly different with isometric exercise. The authors determined that various markers of oxidative stress are associated with different exercise models, and this may be attributable to the differing metabolic demands of each.

It is possible, however, that exercise-induced inflammation can be beneficial although inflammation is strongly associated with increased chronic disease risk. While acute aerobic exercise in untrained animals induces oxidative stress, adaptation with endurance exercise training may increase antioxidant status and reduce oxidant enzymes

(Leeuwenburg & Heinecke, 2001). Similarly, a study conducted by Mataix et al. (1998) observed an increase in glutathione peroxidase, an inherent antioxidant system found in skeletal muscle, after aerobic exercise training. In addition, physically active individuals have lower chronic circulating levels of inflammatory biomarkers, and thus a reduced chronic disease risk over their lifetimes (Gleeson, 2007). However, similar to pro-oxidant effects with high doses of HT, an acute bout of prolonged and sustained aerobic exercise can induce ROS production that surpasses the endogenous antioxidant system, leading to oxidative stress, even in well-adapted, elite endurance athletes (Teixeira et al., 2009). Accordingly, with the right dose of antioxidant(s), there may be a way to combat the oxidative stress resultant of an acute bout of aerobic exercise. It may be possible to increase overall antioxidant status to attenuate a rise in ROS without stimulating a pro-oxidant effect. The following section will discuss how antioxidant supplementation can be used to contest ROS.

Antioxidant Supplementation

Antioxidants reduce ROS damage by significantly reducing the rate of oxidation, usually through donation of an electron to the ROS (Cesari et al., 2004). To slow oxidative stress, endogenous antioxidant systems must be fully developed to protect against destruction of healthy cells and tissues. The endogenous defense systems can be supplemented exogenously from natural as well as synthetic sources. Naturally occurring polyphenols, flavonoids, and vitamins are typically found in fruits and vegetables. Vitamins C and E and other antioxidants including quercetin, epigallocatechin gallate (EGCG), and juice extract from pomegranates and cherries, have been studied to

determine effects on diminishing exercise-related muscle damage and soreness and improving exercise performance. Quercetin is an antioxidant found in the skins of many common fruits and vegetables. Davis et al. (2010) studied aerobic exercise performance with quercetin supplementation and found the supplement to elicit a significant increase in ride time to fatigue and VO_{2max} in fit but untrained young adults. It is hypothesized that the increase in VO_{2max} and ride time to fatigue is attributable to increased mitochondrial biogenesis (Hao et al., 2010). Biogenesis can result in an increased number of mitochondria as well as a denser mitochondrial matrix. This allows for greater distribution of the expected output at a given workload thereby attenuating the leakage of ROS.

In another study, Bigelman et al. (2010) tested six weeks of quercetin supplementation on physical performance in ROTC cadets. The authors measured VO_{2peak} as a potential indicator of increased mitochondria because previous research showed an increase in mRNA expression of SIRT1 and PGC-1α, both co-activators of mitochondrial biogenesis, an increase in mitochondrial density, and increase in run time to exhaustion in mice (Davis et al., 2009). Unfortunately, the authors found no significant increase in VO_{2peak}, nor other measures of physical performance: the Army Physical Fitness Test, Baumgartner Modified Pull-Up Test, Wingate Anaerobic Test, and a 36.6-m sprint.

Cureton and colleagues (2009) had similar findings. Thirty recreationally active young men were assigned to either placebo or quercetin supplementation for a minimum of 7 days. Subjects performed a VO_{2peak} test and a 10 min cycling test for maximum

work output, which was then followed by one hour of submaximal cycling. The authors found no statistically significant difference in VO_{2peak} or performance measures between supplement groups in young, untrained men, therefore, no observable increase in muscular oxidative capacity.

To measure the benefits of antioxidants on the human body, it is necessary to understand the mechanism of action. Important questions to be raised: Are the cells and tissues utilizing the antioxidants? And are the antioxidants combating the ROS? Measurements of serum and plasma markers can identify the effectiveness of such antioxidant supplements.

Measures of Oxidative Stress

Oxidative stress due to ROS assault can be examined by measuring internal antioxidant defenses and levels of inflammation. For example, antioxidant status and uric acid levels indicate activity levels of endogenous antioxidant defense systems. Oxidized low-density lipoprotein (oxLDL), C-reactive protein (CRP), isoprostanes, and cytokines including interleukins and TNFα are indicators of inflammation and inflammatory response mechanisms. Each of these markers will be further discussed.

Antioxidant Status

As briefly discussed, antioxidant status may increase in response to an aerobic exercise stimulus, an antioxidant supplement, or a combination of both. A study conducted by Gonzalez-Santiago et al. (2006) demonstrated an increase in antioxidant status when the diet of rabbits was supplemented daily with 4 mg of hydroxytyrosol/kg body weight. A group of 8 rabbits was fed an HT supplement along with a standard

rabbit diet for one month (group H). Another group of 8 rabbits was fed an atherogenic diet, high in cholesterol and saturated fat, for one month followed by one month of a standard rabbit diet and HT supplementation (group AH). Both groups (H and AH) showed a significant increase in total antioxidant capacity using Trolox as a standard (TEAC) when compared to the relevant control groups of 8 rabbits each that consumed a standard rabbit diet for one month (group C) or two months (group CC), respectively.

Using an exercise model, Berzosa et al. (2011) found a significant increase in plasma TEAC after acute exercise bouts in healthy untrained males. Thirty-four subjects performed three different cycle ergometer exercise protocols in random order with at least one week between each test: VO_{2max}, ride to exhaustion, and a timed submax test. In the continuous progressive protocol, maximal oxygen consumption (VO_{2max}) and maximal working capacity (MWC) were determined by increasing the workload by 10 Watts every minute. The strenuous protocol required subjects to ride until exhaustion at VO_{2max}. Last, the subjects performed a submaximal test at 70% of expected maximum for 30 min. Blood drawn immediately after all three of the cycle ergometer exericise protocols exhibited significantly higher total antioxidant values compared to basal level. The researchers concluded that a redox-sensitive pathway contributes to the increase in enzymatic and non-enzymatic antioxidant defenses after acute exercise.

On the other hand, Morillas-Ruiz et al. (2006) did not find a significant increase in TEAC with exercise nor in combination with an antioxidant supplemented beverage. Trained cyclists performed two exercise bouts for 90 minutes at 70% VO_{2max} on different occasions separated by one week. Subjects were randomly assigned to consume

throughout the exercise bout 1600 ml of either a placebo beverage or an antioxidant supplemented beverage consisting of anthocyanins, flavonols, hydroxycinnamic derivatives, stilbenes, and ellagic acid totaling 1253.5 mg antioxidants/L. There was a significant increase in creatine kinase (CK) and lipid oxidation, as measured by thiobarbituric acid reactive substances (TBARS), which showed the exercise stimulus to be sufficient to elicit muscle damage and oxidative stress; however, there was no significant difference in TEAC resulting from exercise or the test beverage consumed. The authors of this study concluded that exogenous antioxidants contribute little to overall antioxidant status with a good portion of the increase attributed to increased levels of uric acid after strenuous aerobic exercise.

URIC ACID

Uric acid is a by-product of purine metabolism and is described as a non-enzymatic antioxidant that may protect against oxidative damage by contributing to antioxidant defenses through donation of electrons (Berzosa et al., 2011; Halliwell et al., 1995; Powers et al., 2004; Powers & Jackson, 2008). As previously discussed, strenuous exercise can increase levels of uric acid. Mastaloudis et al. (2001) observed this effect in trained runners during a 50 km ultramarathon. Uric acid was measured immediately post-race and found to be significantly higher than the pre-race values. In contrast, Quindry and others (2003) found uric acid levels to be significantly lower immediately following maximal exercise in healthy, untrained male subjects.

Similar to total antioxidant status in plasma, uric acid levels have the potential to be altered with exogenous antioxidant supplementation. For example, uric acid was

significantly decreased in rabbit plasma after nine-weeks of consumption of a standard diet with the addition of 10% extra virgin olive oil or the addition of 10% extra virgin olive oil and 7 mg/kg of oleuropein when compared to the control group consuming a standard diet. Researchers concluded that the presence of exogenous antioxidants performing similar functions as the endogenous antioxidant defenses decreases the need for metabolic production of uric acid (Coni et al., 2000). Conversely, uric acid values were not significantly affected with daily quercetin supplementation over a two-week period in healthy, non-exercising individuals (Egert et al., 2008). Thus, uric acid levels and total antioxidant status gives some indication to the viability of internal defenses against oxidative stress and inflammation.

OXIDIZED LOW-DENSITY LIPOPROTEIN (OXLDL)

A valuable marker of oxidative stress is oxLDL. High levels of oxLDL have been linked to instances of atherogenesis because it is a contributor to the atherogenic process. Once LDL particles become oxidized, they become chemo-attractants for monocytes. Monocytes and leukocytes enter the arterial intima where the oxidized LDL is engulfed by macrophages creating a foam cell. The foam cells cluster together to form a "fatty streak," an indication of advanced athersclerosis (Patrick & Uzick, 2001). LDL particles that have been oxidized are more atherogenic than ordinary LDL, and inhibition of LDL oxidation has been shown to slow the progression of atherosclerotic lesions in the endothelium (Manna et al., 1997). The LDL particle contains internal protection mechanisms through lipid composition of the lipoprotein particle and the concentration of antioxidants bound to it (Masella et al., 2001). For example, MUFA consumption

produces oleate-rich LDL, which is more resistant to oxidative modification (Ferrara et al., 2000).

In addition to MUFA consumption, dietary antioxidant intake can inhibit LDL oxidation by scavenging potentially damaging free radicals, chelation of transitional metal ions, and protection of antioxidants within the LDL particle. Many studies have demonstrated a decrease in circulating oxLDL levels, an increase in the number of phenolic compounds bound to the LDL particle, and the extent of LDL oxidation when consuming olive oil with a higher phenolic content (Covas et al., 2006; EFSA Panel 2011; Fito et al., 2005; Fitó et al., 2007; Raederstorff, 2009; Rietjens et al., 2007; Tuck & Hayball, 2002; Weinbrenner et al., 2004). In a study by Vazquez-Velasco et al. (2010), healthy volunteers between 20 and 45 years of age were randomly assigned to one of two treatments for 3 weeks and then crossed over to the other treatment for 3 weeks after a 2 week washout period. The treatments consisted of a placebo of 10-15 g/day of sunflower oil and the other treatment was the placebo enriched with 45-50 mg/day of HT. When the sunflower oil was combined with HT, oxidized LDL levels were significantly lower compared to the sunflower oil.

As demonstrated with the previous *in vivo* study, HT is also a powerful inhibitor of LDL oxidation *in vitro* (Aruoma et al., 1998; Aviram & Fuhrman, 1998). Aruoma and colleagues (1998) examined the effects of oxidation on LDL particles when incubated with a peroxyl radical and HT. An EDTA-free LDL solution was incubated with 2,2'-azobis(2-amidinopropane) hydrochloride (AAPH), a peroxyl radical, and varying amounts of HT. Absorbance at 234 nm was measured over time, using a

spectrophotometer, and an inverse dose-response relationship was observed in the medium, such that a higher concentration of HT (0 μM, 1 μM, 2.5 μM, 5 μM, and 10 μM) exhibited lower levels of peroxyl radical destruction of the LDL particle. In addition to oxLDL, another marker of oxidative stress and inflammation is C-reactive protein.

C-REACTIVE PROTEIN (CRP)

CRP is an innate immune system recognition and effector molecule produced in the liver as the result of an acute-phase response and can increase from less than 1 μg/ml to as high as 1,000 μg/ml. CRP synthesis is stimulated by inflammatory cytokines, such as TNFα, IL-6 and IL-1 and circulating levels of CRP typically correlate with IL-6 levels, both markers indiciative of inflammation (Du Clos & Mold, 2004; Vincent & Taylor, 2006).

More importantly, circulating levels of CRP are closely linked with disease risk. Elevated CRP levels are related to increased risk of death associated with coronary heart disease, coronary artery disease, and myocardial infarction in patients with angina pectoris (Patrick & Uzick, 2001). Participants in the Women's Health Study were 4.4 times more likely to suffer a cardiovascular event in the highest quartile of high sensitivity CRP (hs-CRP), which was the most sensitive marker of cardiovascular events in this study. As such, participants in the highest quintile of both hs-CRP and the ratio of total cholesterol to HDL exhibited an 8.7 fold increased risk for a cardiac event (Patrick & Uzick, 2001).

CRP levels may be attenuated with regular exercise or antioxidant supplements. For instance, participants with a higher risk ratio of myocardial infarction assigned to a

six-month individualized, supervised exercise program were evaluated for CRP levels before and after the treatment period. Although not significant, there was a 35% decrease in serum CRP levels after training. The authors did find, however, a signficant decrease in atherogenic cytokines and a significant increase in atheroprotective cytokines suggesting that long-term exercise decreases atherogenic activity of blood mononuclear cells and protection against ischemic heart disease (Smith et al., 1999). Furthermore, Fito et al. (2008) observed a significant decrease in CRP after chronic consumption of virgin olive oil. Stable coronary heart disease patients were initially randomized to one of two treatments: 50 ml of virgin olive oil or 50 ml of refined olive oil daily for three weeks, and then crossed-over to the other group after a 2 week washout period. Compared to refined olive oil consumption, both CRP and IL-6 levels were significantly decreased after 3 weeks of consuming virgin olive oil. The authors concluded that virgin olive oil consumption could provide beneficial effects by lowering markers of inflammation in this population.

ISOPROSTANES

F_2-isoprostanes are another marker of oxidative stress, as they are a reliable indicator of lipid peroxidation (Mastaloudis et al., 2001; Raederstorff, 2009; Vincent & Taylor, 2006). A reduction in excreted isoprostanes is evidence of decreased lipid peroxidation in healthy humans (Schwedhelm et al., 2003). An inverse dose-response relationship exists between the rate of excretion of an isoprostane, specifically 8-iso-PGF_{2a}, and amounts of phenolics, or HT, ingested (Cornwell & Ma, 2008; Covas, 2007; (Tuck & Hayball, 2002; Visioli et al., 2000; Visioli et al., 2001). On the contrary, Leger

et al. (2005) did not see a significant variation in 8-iso-PGF_{2a} urinary exretion during four-days of HT supplementation. The dose of HT administered during this protocol is typical of a southern European diet and approximately equivalent to consuming 15 olives on the first day followed by consumption of 7-8 olives per day for the remaining three days. The subjects in this study were Type I diabetics whose basal 8-iso-PGF_{2a} levels were already elevated three-fold; therefore, the authors attributed the lack of a significant decrease in isoprostane excretion to the elevated basal levels.

F_2-isoprostane excretion can also be the result of an acute exercise stimulus. For example, Mastaloudis et al. (2001) observed a significant increase in F_2-isoprostane excretion after strenuous exercise. F_2-isoprostane levels of 11 trained endurance runners were measured before a 50 km ultramarathon and immediately after the race. The levels of F_2-isoprostane increased significantly from pre-race to post-race when compared to a sedentary protocol one month later, suggesting that extreme endurance exercise increases lipid peroxidation and F_2-isoprostanes. The increased oxidative stress due to exercise can also be exhibited by release of various cytokines as described below.

CYTOKINES

Cytokines are potent intercellular signaling molecules that modulate inflammatory and immune responses (Nieman et al., 2005). During the acute phase response, levels of TNFα, IL-6, and IL-1 are increased, and consistent elevation of these cytokines is strongly correlated with risk of primary and subsequent myocardial infarction and death (Patrick & Uzick, 2001). As previously discussed, CRP production is modulated by

TNFα, IL-6, and IL-1; however, IL-10, an anti-inflammatory cytokine, is stimulated by CRP (Du Clos & Mold, 2004).

In healthy subjects, acute exercise increases the plasma concentrations of pro-inflammatory cytokines, specifically TNFα and IL-1β; however, a significant increase in these inflammatory markers may be attenuated by the release of anti-inflammatory cytokines: IL-10, and interleukin-one receptor antagonist (IL-1ra) (Baydur et al., 2010; Nieman et al., 2001). Muscle contractions during aerobic exercise release and significantly increase IL-6 levels, and this increase in IL-6 can suppress the production of TNFα during an acute bout of exercise (Gleeson, 2007; Teixeira et al., 2009). Suzuki et al. (2000) showed the effects of exercise on circulating cytokine levels. IL-1ra and IL-6, both negative feedback inhibitors of cytokine production, and IL-10, an inhibitory cytokine, all significantly increased during a marathon race in trained endurance runners. Nieman and others (2005) observed similar findings in trained runners after a 160 km race.

Antioxidant supplementation during exercise causes equivocal results in downgrading cytokine expression related to exercise. For example, increases in inflammatory cytokines, IL-1β, IL-6, and TNFα, were observed after a baseline exercise test consisting of 45 min of aerobic exercise at 70% VO_{2max} on a cycle ergometer in six, healthy untrained males. Post exercise values were significantly attenuated after 1 month of chronic consumption of an antioxidant supplement consisting of vitamins A, C, and E, allopurinol, and *N*-acetylcysteine (Vassilakopoulos et al., 2003).

On the other hand, a study conducted by Teixeira et al. (2009) found no reduction in IL-6 levels after 4 weeks of antioxidant supplementation. Trained kayakers performed a baseline 1000 m simulated kayak race and were then randomly assigned to either a placebo or antioxidant supplement consisting of α-tocopherol, vitamin C, β-carotene, lutein, selenium, zinc, and magnesium for four weeks. After 4 weeks of consumption, the kayakers would then perform the same exercise protocol. Measurements of IL-6 were taken at rest before commencement of the exercise and 15-minutes after the simulated kayak race at both exercise trials. IL-6 increased significantly from rest to post-race regardless of treatment. The authors concluded that this antioxidant cocktail did not offer protection against exercise-induced inflammation as measured by IL-6.

Summary

The lower incidences of cardiovascular disease and cancer associated with the Mediterranean diet have motivated researchers to investigate its essential benefits. Many have found that olive oil, the main source of fat in this diet, is an important contributor to the decreased disease risk. First, olive oil contains a high concentration of monounsaturated fatty acids, which exhibit greater protective properties against free radical damage compared to polyunsaturated fatty acids. Second, olive oil contains a high amount of phenolic antioxidants. Of the three main phenols in olive oil, hydroxytyrosol is more abundant and more powerful because of its high ROS scavenging capability. Many researchers have demonstrated reduced inflammatory markers and thus, a decreased disease risk with chronic consumption of hydroxytyrosol.

Researchers have also investigated the effects of antioxidants, and HT specifically, on aerobic exercise-induced oxidative stress. The high-energy demand required by exercise causes the release of many ROS, which harm surrounding cells and tissues. However, adaptation to exercise increases the endogenous antioxidant systems to combat ROS released from the mitochondria. The results of many studies examining antioxidant consumption in conjunction with aerobic exercise remain inconsistent regarding performance, antioxidant status, and inflammation. At the present time, further research is necessary to elucidate the effects of HT on the specific markers of oxidative stress and inflammation associated with aerobic exercise.

APPENDIX A - INFORMED CONSENT TO PARTICIPATE IN RESEARCH

Informed Consent to Participate in Research

The University of Texas at Austin

IRB #2009-09-0101

You are being asked to participate in a research study. This form provides you with information about the study. The Principal Investigator (PI, the person in charge of this research) or his/her representative will provide you with a signed and dated copy of this form to keep for your reference, and will also describe this study to you and answer all of your questions. Please read the information below and ask questions about anything you don't understand before deciding whether or not to take part. Your participation is entirely voluntary and you can refuse to participate or discontinue participation without penalty or loss of benefits to which you are otherwise entitled. It is important that you be completely truthful with the PI and study staff regarding your health history so that you do not harm yourself by participating in the study.

Title of Research Study:

Effects of water-soluble olive extract supplementation on antioxidant status, exercise performance, muscle metabolism, muscle damage and inflammation

Principal Investigator(s) (include faculty sponsor), UT affiliation, and Telephone Number(s):

John Ivy, Ph.D., Principal Investigator, Department Chair of Kinesiology and Health Education, 512-471-8599

Lynne Kammer, Co-investigator, Research Assistant, Department of Kinesiology and Health Education, 512-471-8601

Zhenping Ding, Co-investigator, Research Engineer, Department of Kinesiology and Health Education, 512-471-8601

Mark Hutchens, M.D., Co-investigator, Physician, 512-473-0201

Funding source (Sponsor):

DSM Nutritional Products (Kaiseraugst, Switzerland)

What is the purpose of this study?

The purpose of the proposed study is to compare the effect of two different doses of an investigational olive extract versus a placebo on exercise performance, muscle metabolism, muscle damage, antioxidant status and inflammation. An investigational olive extract means that it is experimental and not currently marketed for sale. About 60 people will be participating in this study.

What will be done if you take part in this research study?

After the Screening Visit, participation in this research study includes 12 study visits (1 Familiarization Visit, 2 Muscle Biopsy Visits, 2 Blood Draw Visits and 7 Exercise Visits) and one Exit phone call that will occur over 9 weeks. You will also be contacted during the study with reminder emails. The visits are scheduled in advance and must not be changed. If you become sick or have schedule conflicts, we may not be able to accommodate the schedule change while maintaining the protocol test cycles.

You will be randomly assigned one of three supplements: 50mg olive extract, 150mg of olive extract or a placebo that you will consume daily for 6 weeks of the study. You have an equal chance of being assigned to a particular supplement. You will also complete a daily online survey that will take approximately 5 minutes per day. The study will require about 25 hours of your time to complete over 9 weeks. Approximately 60 people from this site will participate in this study.

During the study we will ask you to limit your intake of wine to no more than 2 glasses per week, and coffee and tea to no more than 1 cup per day. We will also provide a list of foods for you to avoid during the 24 hours prior to your Exercise visits (coffee, tea, chocolate, wine, cherry tomatoes, broccoli, blueberries and onions).

We will give you instructions to prepare for each visit to our lab and will send reminder emails to you. Depending on the visit, you must fast (only water) for 2-12 hours. If these instructions are not followed and we cannot reschedule you, you will be excused from the study.

A summary of the tasks by week is in Table 1 and described below:

Familiarization Visit (2-hour fast, 1-hour duration). We will familiarize you with the cycling ergometer and practice breathing into the mouthpiece for the monitoring of oxygen consumption and carbon dioxide emission. We will draw 4ml of blood to send to a lab to verify that you do not have Hepatitis B or C. This practice ride will be shorter than the actual test ride and will take approximately 30 minutes to complete. The results from your Hepatitis B/C test will be available prior to your first Muscle Biopsy Visit. If the test is positive, you will be notified by the study physician to consult with your physician to determine the appropriate course of treatment action, and excused from the study.

Muscle Biopsy Visit (12-hour fast, 1-hour duration). We will perform biopsies at the start and end of the project. Prior to the visit, you will fast for 12 hours (water only) and shave both of your thighs all of the way around your leg.

After you arrive in the lab, 15ml (about 1 tablespoon) of blood will be drawn from a forearm vein. The blood samples will be analyzed for markers of immune system function, muscle damage and general health.

After the blood draw, biopsies (100 mg total - about the size of 4 grains of rice) will be taken by John Ivy, Ph.D. from the vastus lateralis, a thigh muscle. This biopsy procedure will involve first cleaning the skin where the incision will be made and then anesthetizing or eliminating sensation in a two-inch square area on the thigh by injecting a medication (Lidocaine) into the skin using a needle. Next, a 5-8 mm (0.25 - 0.33 inch) incision will be made in the skin and fat below the skin, so that a special needle can be

inserted into the thigh muscle and remove a small piece of muscle. The incision will be closed with a butterfly bandage and covered with multiple layers of gauze covered tightly with tape. We will provide instructions to you to take care of the incision.

Your muscle tissue will be used to analyze changes that may affect your ability to process oxygen during exercise. A portion of it will be sent to the sponsor (DSM Nutritional Products) to determine the effect of the olive product on the gene expression profile involved in the energy metabolism. The sponsor or the investigator will not be performing genetic finger printing and will destroy any remaining tissue after their testing has been completed.

Following the muscle biopsy, we will perform a Dual Energy X-Ray Absorptiometry (DEXA) scan to measure your body composition – specifically, your amount of body fat and lean tissue. During the DEXA scan you will wear clothing that does not contain metal, such as workout shorts and a t-shirt, and lie on a padded table. The DEXA machine is not enclosed like an MRI machine.

VO_{2MAX} and Lactate Threshold (LT) Visit (2-hour fast, 1½-hour duration)**.** We will initially perform a lactate threshold test then a VO_{2MAX} on the bicycle ergometer. You will breathe continuously through a mouthpiece so that we can collect information about your oxygen consumption. The LT test is not exhausting and will consist of a series of 5 min stages for at least 5-6 stages until an increase in baseline blood lactate level is observed. Blood lactate will be measured by a finger prick during the last minute of each stage. Upon completion of the LT test, you will have a 5-minute break where you can drink water, then you will perform a VO_{2MAX}.
During the VO_{2MAX} test, a computer will increase the exercise intensity until muscle fatigue and exhaustion. This test will last approximately 25 minutes and physically stress and challenge you for about 5 minutes at the end of the test.

Steady-State ride with Time Trial (SS-TT) (10-hour fast, 2-hour duration). When you arrive at the lab, we will weigh you, then insert a catheter fitted with a three-way stopcock under sterile conditions, into a forearm vein, then add a catheter-extension, and tape it into place. This will be performed under John Ivy, Ph.D., an exercise physiologist. Ten milliliters of blood will then be drawn. We will draw blood 2 additional times during your ride for a total of 27ml, or about 2 tablespoons, of blood. You should be riding for 1-1.25 hours total time. At the end of the SS-TT test, we will provide a light snack and beverage to you.

Blood Draw. This visit will take approximately 30 minutes. We will draw 15ml of blood from a forearm vein and perform safety blood analyses at weeks 2, 4 and 6 to compare against your baseline blood sample collected at the first Biopsy Visit. The lab results will be available within 48 hours. If the study physician decides that changes in your blood results are due to the study product, we will contact you and ask you to stop consuming the study product. If you have missed more than one dose per week of the Study Product or do not take the Study Product in the morning, you will be excused from the study.

Food and Medication Logs (FM Logs). We will provide you with Food and Medication Logs to record food, drinks, supplements and medication for the days immediately prior

to your Biopsy and Exercise visits. You will be instructed to write down everything in pen on the Food Log that you eat and drink, and any medications and dietary supplements on the Medication Log. You will bring your food logs to your Biopsy and Exercise Visits. We will make copies of your logs and return them to you to help you maintain a consistent diet before your Biopsy and Exercise Visits.

Study Product (SP). You will be randomly assigned one of three supplements (50mg olive extract, 150mg olive extract or placebo). Each supplement consists of 3 similar-appearing capsules per dose. We will not know which supplement you are receiving. We will provide 2 weeks of Study Product to you. Each day you will push all 3 capsules from a blister and take with water when you eat your breakfast. You must take all 3 capsules in the morning. If you miss a dose, do not double the dose or take it in the afternoon. If you miss more than 1 dose per week of the study, you will be excused from the study. If you travel, you must take the Study Product with you to avoid missing a dose. Each time that you come into the lab, you must bring the empty and partially empty packages of Study Product with you; do not throw them out.

Table 1. *Example schedule for this study protocol (continued on next page)*

SP = Study Product
SS-TT = Steady-State/Time-Trial ride
FM Log = Food/Medication Logs
DM = Diet Management
Log = Online Log
LT/VO_{2MAX} = Lactate Threshold and VO_{2MAX} Tests
Fast = no food, coffee, tea or other drinks; only water

Week Location	Mon	Tues	Wed	Thurs	Fri	Sat	Sun
1 Lab		Familiariza-tion (2hr fast)					FM Log
2 Lab		Biopsy, DEXA, Blood *(12hr fast*)	LT/VO_{2MAX} *(2hr fast)*	SS-TT, Blood *(10hr fast*)			
2 Home	FM Log	DM, FM Log	DM, FM Log, Ensure (10hrs before SS-TT)		SP, Log	SP, Log	SP, Log
3 Home	SP, Log	SP, Log	SP, Log	SP, Log	SP, Log	SP, Log	SP, Log
4 Lab				Blood *(12 hr fast)*			
4 Home	SP, Log	SP, Log	SP, Log	SP, Log	SP, Log	SP, Log	SP, Log
5 Lab			SP, Log, LT/VO_{2MAX} *(2hr fast)*	SP, Log, SS-TT, Blood *(10hr fast)*			
5 Home	SP, Log, FM Log,	SP, Log, DM, FM Log	DM, FM Log, Ensure (10hrs before SS-TT)		SP, Log	SP, Log	SP, Log
6 Lab				Blood *(12 hr fast)*			
6 Home	SP, Log	SP, Log	SP, Log	SP, Log	SP, Log	SP, Log	SP, Log
7 Home	SP, Log	SP, Log	SP, Log	SP, Log	SP, Log	SP, Log, DM, FM Log	SP, Log, DM, FM Log
8 Lab	Biopsy, DEXA, Blood *(12hr fast)*	SP, Log, LT/VO_{2MAX} *(2hr fast)*	SP, Log, SS-TT, Blood *(10hr fast)*	SP, Log, SS-TT, Blood *(10hr fast)*			
8 Home	SP (1.5hrs before biopsy), Log, DM, FM Log	DM, FM Log, Ensure (10hrs before SS-TT)	DM, FM Log, Ensure (10hrs before SS-TT)		Log	Log	Log
9 Home	Exit (via phone)						

The Project Duration is: January 2010 to December 2010. It will require 25 hours of your time to complete the study.

What are the possible discomforts and risks?
The investigators have made every effort to keep the risks and discomforts to a minimum. You will be carefully screened at the beginning of this study to see if you can participate safely; however, participation in this study involves some risk.

Muscle Biopsies: Biopsies will be taken by John Ivy, Ph.D., from the vastus lateralis, a quadriceps muscle. You may experience mild discomfort at the site of the incision and biopsy. There may be slight bruising to the skin and the muscle. In approximately one out of every five cases, a general soreness may persist for 2 to 3 days, but this does not inhibit function. In very rare cases, you may experience severe pain. There is a small possibility (1 in 1,300) that the feeling in the biopsy area will be temporarily lost for 2 to 3 months due to damage to the sensory nerves. There is also a possibility of infection, but this can be remedied with the appropriate antibiotics. There are no alternative methods for obtaining muscle samples which are both useful for this study and reduce potential risk. The chance of infection will be minimized by adhering to sterile procedures. The investigators will examine biopsy sites the following day prior to the LT/VO_{2MAX} test.

Blood Draws: In this study a trained technician will obtain a 15-27ml (about 1-3 tablespoons) sample of your blood during each visit that will be analyzed to measure markers of immune system function, muscle damage and general health or metabolism. Blood samples will be obtained by finger-stick, venipuncture and catheterization. A finger-stick at the tip of a finger is routinely used to obtain a small amount of blood for lactate testing. It may be accompanied by minor discomfort, and there is a possibility of infection. Venipuncture involves inserting a needle into a vein in the arm and withdrawing a sample of blood. It is routinely used to obtain blood for physical examinations. Venipuncture is accompanied by minor discomfort at the site of the needle entry and may result in slight bruising and a feeling of faintness. Venipuncture will be used during the Biopsy and Blood Draw Visits. Catheterization of a vein is a routine and preferable procedure for obtaining multiple blood samples because it requires the skin to be pierced only once, since the catheter is left in the vein. Catherization will be used during the Exercise Visits. Some participants may feel faint during catheterization and blood draws. There is the possibility of an infection with catheterization. There are no alternative methods to obtaining blood samples that are both useful to this study and reduce the possible risks.

VO_{2MAX} tests: Near the end of your VO_{2MAX} tests you should feel fatigued and this feeling may last approximately 15 to 20 minutes after the test. You may also feel some stomach discomfort and lightheadedness. However, the likelihood of this is rather remote since you are physically active and normally participate in aerobic exercise.

Steady-State Time-Trial tests: Since you will perform the tests after a 10-hour overnight fast, you may experience symptoms of low blood sugar, or hypoglycemia. These symptoms may include dryness in your mouth, dizziness, feeling faint and disorientation. Performing

the tests after an overnight fast is the best way for us to isolate the effects of our supplement.

DEXA Scans: DEXA scans produce an amount of radiation of 0.02-0.05 mRem, which is similar to 1/10 of a chest x-ray. There is no immediate short-term or long-term risk to adult humans from exposure to this radiation dose. All DEXA scans will be performed by a certified technician.

The intervention being studied involves ingesting an investigational olive product. The olive product is a natural product as it is a water-soluble olive extract derived from olive processing. This kind of product has been given to other persons in a lower concentration and has been tested at significantly higher doses in rodents. The investigational product has been tested in a complete safety package required to test products in human studies. The maximum dose in this study has been safety approved by several safety studies. Health risks related with the intake are not known but not expected. We will carefully monitor your health status during the study.

In addition, we ask that you please consider the 9-week time commitment for this study. You should consider the impact of this on your work, academic and personal commitments before you decide to volunteer as a subject for this study.

There may be risks from participating in this study that are unknown. Any new important information, which is discovered during the study that may affect your decision to stay in the study, will be given to you. If you wish to discuss the information above or any other risks you may experience, you may ask questions now or call the Principal Investigator listed on the front page of this form.

What are the possible benefits to you or to others?

There may be no direct medical benefit for participation; however, you will receive information regarding your diet, BMI, fitness level, body composition and blood chemistry results. You will also receive information on the general outcome of the study upon completion of the analysis.

The study will provide a better understanding of the effects of an investigational olive extract that may help other healthy adults obtain better fitness and reduced inflammation through supplementation.

If you choose to take part in this study, will it cost you anything?

No, it will not cost you anything to participate in the study, and you should not incur additional expenses to participate in this study.

Will you receive compensation for your participation in this study?

Yes, to pay you for your time and travel involved with participating in this study, you will receive a total of $500 upon completion of all study visits and formally exiting the study. You will also receive your data along with an explanation of the results. Partial compensation will be based on completion of the Biopsy and Exercise phases:

$75 for baseline 3-day testing (biopsy, VO2 max/lactate threshold, time trial ride)

$75 for mid point testing at 3 weeks (VO2 max/lactate threshold, time trial ride)
$75 for final 6 week testing (same as baseline)
$275 after exit

At the end of the study we will call you to ask questions about how you feel and if you have experienced any health problems. Once we have spoken to you about the study exit, we will process your payment for $500.

If you complete part of the Biopsy and/or Exercise Visits, you will receive compensation based on your last completed Test Session. You will not receive compensation if you only complete the Screening Visit and Familiarization Visit. Payment will be made to you at the end of the study. You will be responsible for any taxes assessed on your compensation.

What if you are injured because of the study?

In the event of a study related injury, please contact the Principal Investigator. The University has no program or plan to provide treatment for research-related injury or payment in the event of a medical problem. The University also has no program or plan for continuing medical care and/or hospitalization for research-related injuries or for financial compensation. However, if injuries occur as a result of study activity, DSM Nutritional Products agrees to pay medical expenses necessary to treat the injury, if you have followed the directions of the study doctor and to the extent you are not otherwise reimbursed by medical insurance. To receive this medical expense reimbursement from DSM Nutritional Products, you may be asked to provide a copy of your medical records related to this injury and/or be examined a physician selected by DSM Nutritional Products. No other compensation will be provided beyond that which is listed in this informed consent. You will not lose any of your legal rights as a research subject by signing this consent document.

If there is a serious adverse event during the study and you are admitted to a hospital, you agree to provide a copy of your *Discharge Summary* to the Principal Investigator and talk to the Study Physician to answer questions specific to your adverse event.

If you do not want to take part in this study, what other options are available to you?

Your participation in this study is entirely voluntary. You are free to refuse to be in the study, and your refusal will not influence current or future relationships with The University of Texas at Austin.

How can you withdraw from this research study and who should you call if you have questions?

You may leave the study at any time for any reason; however, you may be asked to complete certain procedures, for example cooling down after exercise, since withdrawing during these procedures may present safety concerns.

Anticipated circumstances under which participation may be terminated by the investigator without the participant's consent. These include:

1. The study doctor thinks it is necessary for your health and safety.
2. You have not followed study instructions:
    a. *Diet Management.* You eat foods or consume beverages in excess of the study requirements.
    b. *Inconsistent pre-study exercise and/or diet*. To control our experimental conditions, it is critical that your diet is similar for 48 hours and you do not participate in strenuous exercise before Biopsy and Exercise Visits. Changing your diet and exercise pattern during the days leading up to the Biopsy and Exercise Visits will affect the study results.
    c. *Medications.* You begin taking an excluded medication.
    d. *Supplements.* You begin taking an excluded supplement.
    e. *Study Product.* You do not take complete daily doses during the required timeframe or miss more than one dose per week.
    f. *Online Log*. You do not fill out the daily survey to record your Study Product intake and health status.

3. If you are unable to attend scheduled appointments, do not fast prior to Biopsy and Exercise Visits, do not consume Ensure prior to SS-TT Tests or cannot complete the study within the required time.
4. The Sponsor has stopped the study.
5. You no longer meet the qualification requirements.

If you wish to stop your participation in this research study for any reason, you should contact the Principal Investigator: John Ivy, Ph.D. at 512-471-8599. You should also call the Principal Investigator for any questions, concerns, or complaints about the research. You are free to withdraw your consent and stop participation in this research study at any time without penalty or loss of benefits for which you may be entitled. Throughout the study, the researchers will notify you of new information that may become available and that might affect your decision to remain in the study.

If you would like to obtain information about the research study, have questions, concerns, complaints or wish to discuss problems about a research study with someone unaffiliated with the study, please contact the IRB Office at (512) 471-8871 or Jody Jensen, Ph.D., Chair, The University of Texas at Austin Institutional Review Board for the Protection of Human Subjects at (512) 232-2685. Anonymity, if desired, will be protected to the extent possible. As an alternative method of contact, and email may be sent to orsc@uts.cc.utexas.edu or a letter sent to IRB Administrator, P.O. Box 7426, Mail Code A3200, Austin, TX 78713.

AUTHORIZATION TO USE AND DISCLOSE INFORMATION FOR RESEARCH PURPOSES

Federal regulations give you certain rights related to your health information. These include the right to know who will be able to get the information and why they may be able to get it. The Principal Investigator must get your authorization (permission) to use or give out any health information that might identify you.

How will your privacy and the confidentiality of your research records be protected?

Authorized persons from the University of Texas at Austin, the Institutional Review Board, the Sponsor and its delegates and regulatory authorities have the legal right to review your personal health information and the research records collected in this study for the purposes of collecting data, verifying that the data is correct and checking that the study is conducted properly. This information will be used for clinical research and also for seeking approval from domestic and/or foreign regulatory authorities to market the experimental olive extract.

This research project is sponsored by DSM Nutritional Products (Kaiseraugst, Switzerland). "Sponsor" includes any persons or companies that are working for or with the sponsor, or are owned by the sponsor. This sponsoring agency also has the legal right to review your research records.

All data collected will be stored using a code (subject number and initials). It will not be stored by your name; however, during the study, a list will be maintained, linking your code and name. The contact information, master key list, and other materials containing names will be separated from the results and kept in a locked file cabinet. Your health questionnaire will also be kept confidential and stored in a locked office in the Exercise Physiology & Metabolism Laboratory in Bellmont 818. The list and the health questionnaire will be physically destroyed when the sponsor notifies the investigator that the data no longer needs to be retained. Only principal researchers have access to the file, but representatives of the Sponsor may review the files during the study. We will be collecting your name, phone number, and address for contact, scheduling and payment purposes only. If you have not previously been paid by The University of Texas at Austin, we will also collect your social security number for payment purposes only. This information will be kept secured and will be physically destroyed after you are paid. If you become ineligible to participate at any point during the study, the data collected thus far will be kept confidential and stored in a locked file cabinet until we finish data collection for this study.

If in the unlikely event it becomes necessary for the Institutional Review Board to review your research records, then The University of Texas at Austin will protect the confidentiality of those records to the extend permitted by law. Your research records will not be released without your consent unless required by law or a court order. The data resulting from your participation may be made available to other researchers in the future for research purposes not detailed within this consent form. In these cases, the data will contain no identifying information that could associate you with it, or with your participation in any study.

If the results of this research are published or presented at scientific meetings, your identity will not be disclosed.

What if I decide not to give permission to use and give out my health information?

By signing this consent form, you are giving permission to use and give out the health information listed above for the purposes described above. If you refuse to give permission, you will not be able to participate in this research study.

May I review or copy the information obtained from me or created about me?
You have the right to review and copy your health information. If you decide to participate in this study and sign this permission form, you will not be allowed to look at or copy your study information until after the research is completed.

May I withdraw or revoke (cancel) my permission?
Yes, you may withdraw or take away your permission to use and disclose your health information at any time. You do this by sending written notice to the Principal Investigator. If you withdraw your permission, you will not be able to continue being in this study.

When you withdraw your permission, no new health information, which might identify you, will be gathered after that date. Information that has already been gathered may still be used. This would be done if it were necessary for the research to be reliable.

Is my health information protected after it has been given to others?
If you give permission to give your identifiable health information to a person or business, the information may no longer be protected. There is a risk that your information will be released to others without your permission. However, DSM Nutrition Products will take reasonable measures to prevent the unauthorized disclosure of your individually identifiable health information. Information that is collected for research purposes continues to be analyzed for many years; however, blood and muscle tissue specimens will be destroyed after lab analysis has been completed. This authorization will expire in 10 years, or the date when data and specimens will be destroyed or retained without identifiers, whichever is earlier.

A new Federal law, called the Genetic Information Nondiscrimination Act (GINA), generally makes it illegal for health insurance companies, group health plans, and most employers to discriminate against you based on your genetic information. This law generally will protect you in the following ways:

- Health insurance companies and group health plans may not request your genetic information that we get from this research
- Health insurance companies and group health plans may not use your genetic information when making decisions regarding your eligibility or premiums.
- Employers with 15 or more employees may not use your genetic information that we get from this research when making a decision to hire, promote, or fire you or when setting the terms of your employment.

All health insurance companies and group health plans must follow this law by May 22, 2009. All employers with 15 or more employees must follow this law as of November 21, 2009.

Be aware that this new Federal law does not protect you against genetic discrimination by companies that sell life insurance, disability insurance, or long-term care insurance, nor

does it prohibit discrimination on the basis of a genetic disease or disorder that you already know about.

By signing the consent form, you acknowledge that you have voluntarily donated your blood and muscle tissue specimens to The University of Texas at Austin for research purposes. The University has no plans to compensate you for any commercial uses of the products that may be derived from the specimen. The University of Texas at Austin will maintain ownership of the specimen.

Will the researchers benefit from your participation in this study*?*
The researchers are being paid by DSM Nutrition Products to conduct this study. Otherwise, they will not benefit from this study beyond publishing or presenting the results.

Consent to test your blood for HIV and other diseases
If an investigator is exposed to your blood sample through an inadvertent needle stick or during biochemical analysis, you grant permission to have your blood tested for HIV and other blood-borne diseases. If a positive test is found, you will be informed by the Principal Investigator of the study (John Ivy, Ph.D.) of the results in a private setting.

Please review the risks listed in the following table and initial and date each.

	Risk	***Initials***	***Date***
1	I understand that by participating in this study, any data collected about me including personal and health information will be stored using a subject number and my initials.		
2	I understand that by participating in this study, I will provide blood and tissue specimens that will become the property of The University of Texas at Austin.		
3	I understand that by participating in this study, some of my blood and tissue specimens will be sent to the sponsor company, DSM Nutritional Products.		
4	I understand that by participating in this study, there is a chance that my individually identifiable health information may be obtained from my blood and tissue specimens.		
5	I understand that by participating in this study, my health information will be used for investigational purposes only and not disclosed to any outside agency unless required by law or a court order.		
6	I understand that by participating in this study, my personal information, including my social security number if it is not already in The University of Texas at Austin payment system, will be collected so that I may be compensated for my participation as described in this Informed Consent Form. This personal information will be destroyed after you complete the study and have been paid.		
7	I understand that by participating in this study, my blood and tissue specimens, and any data collected about me will be saved for a maximum of 10 years.		

Signatures:

As a representative of this study, I have explained the purpose, the procedures, the benefits, and the risks that are involved in this research study:

Signature and printed name of person obtaining consent **Date**

You have been informed about this study's purpose, procedures, possible benefits and risks, and you have received a copy of this form. You have been given the opportunity to ask questions before you sign, and you have been told that you can ask other questions at any time. You voluntarily agree to participate in this study. By signing this form, you are not waiving any of your legal rights.

Printed Name of Subject **Date**

Signature of Subject **Date**

Signature of Principal Investigator **Date**

APPENDIX B - PARTICIPATION HEALTH RESEARCH SCREENING FORM

Demographic

Today's Date	_____/_____ / _____ *month* *day* *year*
Age:	_____ years
Ethnicity:	☐ Hispanic/Latino ☐ Not Hispanic/Latino
Race:	☐ White ☐ Black/African American ☐ American Indian/Alaskan Native ☐ Asian ☐ Native Hawaiian/Other Pacific Islander ☐ Other

Exercise Habits

Please describe your regular physical activity during a typical week. Please include the type of activity, duration and intensity. For intensity use: ***Low:*** My heart rate did not rise very much, I did not sweat or sweated very little ***Medium:*** My heart rate rose somewhat, but I was able to talk easily ***High:*** My heart rate was very high and I was unable to carry on a conversation		Exercise & Duration	Intensity (L/M/H)
	Mon		
	Tues		
	Wed		
	Thurs		
	Fri		
	Sat		
	Sun		

Answer the questions below by checking the appropriate box.

Yes	No		
☐	☐	1.	Has your doctor ever said that you have a heart condition and that you should only do physical activity recommended by a doctor?
☐	☐	2.	Do you feel pain in your chest when you do physical activity?
☐	☐	3.	In the past month have you had chest pain when you were not doing physical activity?
☐	☐	4.	Have you ever lost your balance because of dizziness or have you ever had a loss of consciousness? If yes explain: _____
☐	☐	5.	Have you ever had racing of your heart or skipped heartbeats?
☐	☐	6.	Has any family member or relative died of heart problems or sudden death before the age of 50?
☐	☐	7.	Have you been told you have high blood pressure?
☐	☐	8.	Have you been told you have a heart murmur?
☐	☐	9.	Have you been told that you have kidney disease?
☐	☐	10.	Have you been told that you have Type 1 or Type 2 diabetes?
☐	☐	11.	Have you received an organ transplant?
☐	☐	12.	Have you had or do you currently have a malignancy (cancer)?
☐	☐	13.	Have you had a severe viral infection within the past month?
☐	☐	14.	Have you been told that you have a chronic contagious, infectious disease such as tuberculosis, hepatitis B, hepatitis C or HIV?
☐	☐	15.	Are you taking any performance enhancing drugs or supplements other than a multivitamin. If so, what? _____
☐	☐	16.	Do you have any medical problems or illnesses such as osteoporosis, diabetes, kidney or liver disease, a chronic contagious or infectious disease? If yes, please describe: _____
☐	☐	17.	Are you currently on any regular medication including non-prescription medications? If your answer is "yes," please list your regular medication(s) and how long you have been taking it (them): _____ _____
☐	☐	18.	Are you currently in another study?

Yes **No**

☐ ☐ 19. Do you have any known allergies? If yes please specify:

_____

20. Approximately how much olive oil to you consume per day?

_____

21. Approximately how much chocolate do you consume per day?

_____

☐ ☐ 22. Do you smoke?

23. When was the last time you had a physical? _____

_____ _____

Signature Date

APPENDIX C – SAMPLE FOOD LOG

MEAL	*Amount*	*Brand*	*Food*
Breakfast			
Lunch			
Dinner			
Snacks			

APPENDIX D – DATA

Data that was not collected is marked with "---". Data is divided by treatment group for each variable.

TEAC

TEAC (mM) Raw Data – PLA (n=19)

Random Number	TT1		TT2		TT3		TT4	
	pre	end	pre	end	pre	end	pre	end
102	0.47	0.45	0.59	0.42	0.53	0.46	0.47	0.49
106	1.14	0.95	1.12	0.78	1.08	1.20	1.32	1.09
107	0.87	0.79	0.94	0.88	0.72	0.67	0.80	0.86
110	1.21	1.23	1.29	1.48	1.22	1.44	1.35	1.13
113	1.01	1.08	1.03	1.04	0.99	1.15	0.99	0.99
117	0.79	1.21	0.84	0.75	0.72	0.83	0.76	0.73
120	1.35	1.16	1.34	1.18	1.28	1.35	1.08	1.24
125	0.94	1.09	1.32	1.21	0.88	1.22	1.22	1.25
126	1.20	1.06	1.18	1.23	1.00	1.25	1.30	1.20
139	0.95	1.10	1.02	0.89	0.82	1.00	1.03	0.93
141	1.34	1.28	1.29	1.19	1.25	1.14	1.40	1.19
147	1.03	1.14	---	---	0.91	1.08	1.06	1.02
148	0.87	0.73	0.78	0.79	0.94	1.10	0.80	0.86
149	1.60	1.51	1.47	1.28	1.26	1.18	1.61	1.48
153	1.03	1.16	1.08	1.13	0.84	0.94	0.63	1.12
157	0.84	0.94	1.06	1.38	0.69	0.70	0.84	0.77
160	0.51	0.47	0.40	0.42	0.37	0.42	0.49	0.45
161	1.26	1.16	1.54	1.56	1.58	1.34	1.77	1.49
171	1.16	1.13	1.15	1.08	1.00	1.13	1.25	1.16
Mean	**1.03**	**1.03**	**1.08**	**1.04**	**0.95**	**1.03**	**1.06**	**1.03**

TEAC (mM) Raw Data – LHT (n=20)

Random Number	TT1		TT2		TT3		TT4	
	pre	end	pre	end	pre	end	pre	end
101	0.73	0.79	0.65	0.82	0.66	0.73	0.79	0.73
104	1.14	0.94	1.29	1.15	0.69	1.37	0.98	1.03
108	0.72	0.90	0.97	0.95	0.81	0.78	0.87	---
111	1.16	1.21	0.89	1.31	1.24	1.07	1.19	1.17
114	0.62	1.09	0.95	0.71	0.66	0.91	0.76	0.71
115	0.80	0.88	1.04	0.94	0.99	0.98	0.88	0.81
127	0.90	1.02	1.05	0.98	0.86	1.13	1.07	1.16
129	1.14	1.15	1.14	1.08	0.91	1.30	1.40	1.13
132	1.08	1.02	1.13	1.03	0.99	1.04	1.29	1.27
136	0.71	0.76	0.67	0.76	0.67	0.87	0.92	0.84
137	1.05	0.98	0.96	0.79	0.79	1.10	0.96	0.96
140	0.77	0.88	0.79	0.79	0.72	0.79	1.08	0.74
143	1.19	1.15	1.51	1.19	1.30	1.70	1.58	1.83
146	1.21	1.40	1.23	1.15	1.06	1.13	1.08	1.29
150	0.98	1.07	0.74	0.92	0.90	1.05	1.23	0.99
151	0.79	0.71	0.71	0.85	0.88	0.77	0.88	1.06
155	0.29	0.52	0.69	0.65	0.72	0.54	0.61	0.60
158	0.91	0.81	1.23	1.03	1.04	0.73	1.04	1.04
162	1.43	1.16	1.16	0.97	1.08	1.37	1.32	1.03
166	0.85	0.76	1.00	0.93	0.86	1.04	0.86	1.07
Mean	**0.92**	**0.96**	**0.99**	**0.95**	**0.89**	**1.02**	**1.04**	**1.02**

TEAC (mM) Raw Data – HHT (n=22)

Random Number	TT1		TT2		TT3		TT4	
	pre	end	pre	end	pre	end	pre	end
103	0.54	-0.22	0.52	0.55	0.47	0.52	0.59	0.56
105	0.98	0.85	1.20	1.04	1.15	0.80	0.80	0.98
109	1.04	0.89	1.14	0.91	0.69	1.12	1.13	0.80
112	1.09	1.08	1.01	0.98	0.82	0.85	0.98	0.99
116	0.77	0.85	0.87	0.81	0.73	0.87	0.89	0.86
118	1.14	1.01	1.14	1.21	0.96	1.21	1.39	1.21
122	0.77	0.92	0.99	1.23	1.31	0.98	1.06	1.09
123	0.89	1.24	1.24	1.22	0.98	1.02	0.98	1.22
128	1.10	1.01	1.28	1.25	1.23	0.92	1.02	1.24
130	1.02	1.12	1.05	1.23	0.90	1.00	0.77	1.13
131	1.34	1.28	1.20	1.12	1.09	1.11	1.26	1.33
133	0.86	0.86	0.86	0.98	0.95	0.93	1.10	0.96
138	1.00	0.69	1.10	0.85	0.85	0.73	0.91	0.93
142	1.12	0.96	0.87	0.94	0.85	1.19	1.27	1.12
144	0.95	0.87	0.90	0.86	0.81	1.03	1.09	1.07
145	1.10	0.89	1.50	1.27	1.30	1.03	1.66	1.25
152	0.85	0.79	0.97	1.07	0.92	0.89	1.03	0.96
154	0.65	---	0.87	0.91	0.85	0.86	0.99	0.84
156	0.73	0.68	0.82	0.77	0.72	0.93	0.66	0.90
159	1.19	0.85	0.69	0.57	0.91	0.96	0.98	0.96
167	0.95	0.94	1.16	1.16	1.22	1.18	0.89	1.32
172	1.03	1.03	0.86	1.05	0.84	1.00	1.09	0.93
Mean	**0.96**	**0.88**	**1.01**	**1.00**	**0.93**	**0.96**	**1.02**	**1.03**

oxLDL

oxLDL (ng/ml) Raw Data – PLA (n=19)

Random	TT1		TT2		TT3		TT4	
Number	pre	end	pre	end	pre	end	pre	end
102	112.63	175.55	125.21	92.22	66.61	90.93	61.59	58.56
106	31.07	25.33	41.65	23.95	35.20	36.04	29.99	41.03
107	108.74	89.34	94.45	102.01	102.01	120.67	97.19	113.22
110	11.29	26.13	30.01	24.46	23.10	17.07	18.75	29.86
113	111.75	111.06	112.09	115.18	119.31	114.49	---	113.12
117	550.78	593.02	366.47	503.34	298.65	339.97	205.24	327.86
120	52.19	49.54	49.81	57.56	43.78	41.73	39.01	46.14
125	104.57	131.69	86.31	103.65	74.49	80.10	76.36	54.74
126	9.77	3.96	11.46	15.10	21.65	28.76	20.68	25.22
139	107.93	147.63	86.41	91.18	74.74	127.80	87.61	100.21
141	593.33	767.97	586.13	719.16	503.81	683.17	624.59	784.04
147	108.07	112.05	---	---	94.94	113.48	67.07	109.88
148	168.99	199.02	171.14	209.56	190.18	222.92	166.51	210.98
149	68.12	76.28	63.48	67.42	61.20	69.75	60.53	62.53
153	1910.92	2671.23	1941.63	2510.55	2815.09	2674.43	2386.77	2331.53
157	29.29	37.64	23.61	37.30	25.75	36.61	31.40	29.24
160	33.78	30.76	23.41	19.62	22.89	26.63	27.65	46.21
161	100.78	101.62	91.94	94.03	89.30	108.61	93.62	103.62
171	123.98	148.08	114.63	80.95	74.29	46.60	55.05	88.94
Mean	**228.31**	**289.36**	**223.32**	**270.40**	**249.32**	**262.09**	**230.53**	**246.15**

oxLDL (ng/ml) Raw Data – LHT (n=20)

Random	TT1		TT2		TT3		TT4	
Number	pre	end	pre	end	pre	end	pre	end
101	27.69	28.72	48.72	66.52	363.18	584.40	328.31	505.86
104	122.50	120.54	56.40	106.03	89.20	139.81	109.12	116.03
108	259.56	258.36	245.57	211.36	146.63	244.68	240.22	---
111	266.06	246.57	169.16	210.37	168.12	197.13	166.71	312.47
114	66.22	68.32	52.94	55.51	62.34	62.62	46.37	77.65
115	64.94	83.66	25.31	52.96	38.63	38.72	29.09	54.02
127	29.73	18.48	22.95	21.64	9.45	17.74	8.11	11.11
129	28.70	41.24	32.83	22.04	22.88	26.69	0.00	23.78
132	301.03	285.44	261.92	233.10	242.59	264.69	213.07	287.90
136	70.51	40.10	48.14	41.92	21.93	45.07	41.49	37.33
137	1942.14	1623.35	1811.73	1932.98	1860.17	1745.68	2007.09	2341.54
140	40.07	44.39	32.15	28.75	23.21	28.91	23.07	30.19
143	91.11	100.79	61.04	78.18	77.73	85.56	71.54	89.41
146	11.63	11.38	10.24	11.17	9.33	6.69	10.13	22.06
150	130.80	139.93	89.03	149.67	113.56	113.48	135.36	140.87
151	204.92	214.68	162.25	155.36	167.22	194.29	182.97	228.88
155	118.86	126.85	102.98	109.44	91.64	114.12	105.03	102.10
158	183.23	221.73	125.12	238.72	187.93	225.07	273.40	235.44
162	885.05	606.60	582.13	776.05	453.42	649.36	598.76	600.76
166	95.40	97.51	39.31	33.73	39.67	30.57	19.17	62.43
Mean	**247.01**	**218.93**	**199.00**	**226.78**	**209.44**	**240.76**	**230.45**	**277.89**

oxLDL (ng/ml) Raw Data – HHT (n=22)

Random Number	TT1		TT2		TT3		TT4	
	pre	end	pre	end	pre	end	pre	end
103	13.16	15.11	14.60	12.31	19.48	17.41	11.72	24.38
105	6.76	7.12	4.56	7.53	5.37	6.15	4.39	3.44
109	19.30	13.42	7.48	12.10	24.05	17.19	19.24	19.21
112	24.63	14.74	16.15	16.87	19.36	20.44	19.36	33.76
116	409.40	499.78	425.27	524.61	472.27	512.77	402.92	389.07
118	40.42	66.74	42.48	69.31	73.78	97.26	68.99	57.64
122	334.92	338.47	324.63	319.73	286.50	376.69	305.18	281.22
123	17.39	3.78	4.53	7.83	10.75	0.00	0.00	4.43
128	36.44	21.49	35.48	56.05	37.40	38.67	42.80	49.44
130	398.25	494.54	524.03	568.03	426.11	403.54	448.15	501.74
131	98.39	105.81	95.61	113.19	54.81	95.61	97.09	98.39
133	114.38	119.51	118.38	86.37	122.59	121.62	95.10	88.78
138	934.60	1650.49	1197.20	1306.51	1424.63	1116.44	690.16	1030.76
142	7.19	10.31	12.16	13.23	0.00	1.70	0.00	0.51
144	198.65	230.99	179.19	225.72	205.71	204.87	152.64	200.76
145	36.45	16.95	17.81	16.00	6.34	6.48	23.59	12.42
152	208.94	228.42	180.44	223.78	177.66	247.25	214.20	216.06
154	140.96	---	117.93	142.33	111.40	135.11	136.83	108.66
156	12.90	15.54	14.05	15.70	8.48	15.39	15.48	15.54
159	204.33	200.58	225.06	179.48	95.34	200.58	247.24	417.27
167	2666.57	2398.42	1881.93	2260.42	1142.71	1707.20	1502.60	1526.57
172	35.99	16.22	9.83	14.57	11.76	12.65	8.81	25.25
Mean	**270.91**	**308.02**	**247.67**	**281.44**	**215.30**	**243.41**	**204.84**	**232.06**

CRP

CRP (mg/L) Raw Data – PLA (n=19)

Random	TT1		TT2		TT3		TT4	
Number	pre	end	pre	end	pre	end	pre	end
102	0.51	0.38	0.44	0.57	0.45	0.46	0.79	0.82
106	0.42	0.43	0.33	0.33	0.53	0.60	0.72	0.73
107	0.31	0.32	0.29	0.31	0.34	0.37	0.46	0.46
110	0.23	0.25	0.22	0.23	0.21	0.22	0.24	0.24
113	1.16	1.11	1.29	1.46	0.70	0.88	0.97	1.19
117	2.35	2.54	2.22	2.47	4.34	5.09	4.39	4.87
120	0.59	0.62	3.36	3.58	0.61	0.61	0.58	0.68
125	0.34	0.33	0.36	0.34	0.43	0.40	0.38	0.39
126	0.54	0.60	0.66	0.65	2.53	2.84	1.96	2.03
139	0.38	0.39	0.71	1.06	0.54	0.56	0.77	0.93
141	0.84	0.95	0.51	0.58	1.48	1.74	2.89	3.33
147	6.07	7.21	---	---	0.49	0.56	0.72	0.88
148	1.03	1.34	1.42	1.17	1.11	1.39	1.93	1.96
149	0.99	1.07	1.72	1.89	1.27	1.17	3.65	4.08
153	2.01	2.25	0.97	1.00	1.62	1.71	1.65	2.17
157	0.96	1.29	0.99	0.92	6.81	7.24	9.03	10.23
160	1.39	1.59	0.61	0.66	1.97	1.77	1.53	1.73
161	0.58	0.61	0.50	0.58	0.43	0.43	0.76	0.78
171	0.84	0.88	1.99	2.20	1.63	2.75	5.89	6.21
Mean	**1.13**	**1.27**	**1.03**	**1.11**	**1.45**	**1.62**	**2.07**	**2.30**

CRP (mg/L) Raw Data – LHT (n=20)

Random	TT1		TT2		TT3		TT4	
Number	pre	end	pre	end	pre	end	pre	end
101	0.60	0.68	0.58	0.64	2.13	2.40	2.16	2.46
104	0.33	0.33	10.36	9.80	0.92	0.97	0.62	0.69
108	0.38	0.42	0.35	0.34	1.62	1.68	0.95	---
111	0.44	0.49	0.45	0.53	1.32	1.43	2.50	2.06
114	0.37	0.40	0.50	0.50	0.39	0.43	0.47	0.54
115	0.62	0.68	0.41	0.43	0.36	0.42	0.71	1.00
127	0.56	0.61	0.39	0.45	4.77	4.56	4.09	4.40
129	2.48	2.69	1.23	1.19	1.27	1.51	1.35	1.46
132	0.39	0.43	0.26	0.29	0.35	0.34	0.36	0.42
136	2.79	2.91	1.40	1.47	1.08	0.97	1.07	1.19
137	0.86	0.93	0.85	0.95	1.45	1.60	4.11	4.06
140	0.39	0.42	0.61	0.69	0.69	0.73	0.88	0.93
143	1.02	1.16	0.56	0.59	3.88	4.48	3.32	3.55
146	1.05	1.09	0.76	0.76	1.08	1.32	1.34	1.51
150	0.56	0.59	0.47	0.48	0.85	0.98	1.12	1.22
151	0.38	0.46	5.62	6.48	0.37	0.38	0.42	0.44
155	1.71	2.14	2.33	2.26	2.16	1.97	2.35	2.66
158	1.05	1.19	1.39	1.39	0.71	0.85	1.23	1.34
162	0.33	0.40	0.34	0.37	0.37	0.40	0.42	0.43
166	2.73	2.94	1.97	1.90	4.19	4.48	4.89	5.62
Mean	**0.95**	**1.05**	**1.54**	**1.58**	**1.50**	**1.60**	**1.72**	**1.89**

CRP (mg/L) Raw Data – HHT (n=22)

Random	TT1		TT2		TT3		TT4	
Number	pre	end	pre	end	pre	end	pre	end
103	2.54	2.86	1.58	1.61	4.47	6.18	6.01	6.73
105	4.28	4.99	1.18	1.12	0.66	0.80	1.54	1.95
109	0.50	0.53	0.34	0.36	0.70	0.68	0.88	0.86
112	0.90	1.18	1.47	1.39	0.64	0.70	1.23	1.24
116	0.51	0.45	0.74	0.87	0.46	0.43	1.29	1.43
118	0.30	0.33	0.38	0.41	0.38	0.38	0.75	0.84
122	0.49	0.63	0.40	0.40	0.65	0.75	0.79	0.83
123	2.00	2.27	0.84	0.97	0.96	0.95	0.56	0.71
128	1.24	1.45	0.55	0.69	0.39	0.43	0.47	0.49
130	0.22	0.23	0.22	0.24	0.36	0.43	0.36	0.37
131	0.46	0.53	0.54	0.59	0.42	0.43	0.65	0.74
133	0.67	0.74	0.71	0.82	1.03	1.13	1.52	1.58
138	0.38	0.38	0.75	0.76	0.46	0.49	0.61	0.72
142	1.98	2.48	0.76	0.62	0.76	0.86	1.10	1.19
144	0.39	0.40	0.29	0.31	0.54	0.57	0.46	0.52
145	1.72	1.92	1.74	2.28	1.01	1.34	2.38	2.30
152	2.65	3.60	2.38	2.36	0.96	1.19	1.06	1.22
154	0.83	---	0.74	0.99	0.80	1.15	1.36	1.25
156	0.39	0.41	0.35	0.44	0.37	0.39	0.30	0.34
159	5.82	6.77	4.65	5.84	4.56	6.05	5.84	6.99
167	11.00	12.93	4.79	5.43	0.52	0.52	0.74	0.85
172	0.62	0.60	0.39	0.39	0.44	0.47	0.80	0.84
Mean	**1.81**	**2.18**	**1.17**	**1.31**	**0.98**	**1.20**	**1.40**	**1.55**

8IP

8IP (pg/ml) Raw Data – PLA (n=19)

Random	TT1		TT2		TT3		TT4	
Number	pre	end	pre	end	pre	end	pre	end
102	25.49	38.75	33.00	36.06	36.18	43.08	29.60	27.23
106	32.05	24.70	22.95	40.15	28.40	26.28	23.08	26.13
107	25.73	23.40	20.18	33.13	22.60	28.85	19.53	26.20
110	29.80	25.03	23.98	24.20	32.00	27.58	27.58	24.05
113	26.05	16.40	27.40	18.93	21.03	22.15	17.05	23.40
117	34.15	25.18	24.45	31.35	29.80	34.35	22.95	30.95
120	37.10	26.78	43.26	33.69	36.03	32.36	23.07	32.73
125	30.75	36.96	31.91	38.21	34.37	34.71	34.89	43.40
126	47.58	38.27	33.96	38.39	30.74	40.22	31.59	37.40
139	46.83	41.48	34.38	47.48	44.34	49.64	42.71	55.31
141	45.84	45.96	40.31	41.10	47.58	55.53	48.84	33.96
147	37.23	61.71	---	---	30.36	36.70	50.91	33.56
148	25.20	27.47	33.50	23.58	27.93	14.21	21.30	26.70
149	39.08	48.41	33.93	41.13	41.60	48.63	37.64	41.60
153	54.93	63.55	40.30	27.58	38.23	39.33	24.85	41.33
157	25.25	21.35	20.70	30.33	25.70	22.83	21.13	20.80
160	12.55	10.35	11.70	12.93	10.78	10.95	11.85	9.53
161	49.61	44.97	47.58	56.10	48.08	53.43	44.30	50.93
171	45.48	44.12	44.12	45.72	39.00	49.56	44.12	49.43
Mean	**35.30**	**34.99**	**31.53**	**34.45**	**32.88**	**35.28**	**30.37**	**33.40**

8IP (pg/ml) Raw Data – LHT (n=20)

Random Number	TT1		TT2		TT3		TT4	
	pre	end	pre	end	pre	end	pre	end
101	20.63	18.68	22.23	23.35	30.35	29.03	18.55	24.70
104	21.35	23.80	26.95	20.90	21.68	22.83	19.63	25.48
108	20.98	26.75	34.93	29.18	22.40	27.65	23.18	---
111	17.93	21.35	20.33	23.95	24.70	28.23	18.05	23.40
114	42.93	39.80	43.88	41.08	28.95	34.68	32.15	36.35
115	27.55	28.78	25.90	28.00	25.55	27.85	26.20	31.75
127	22.98	31.68	29.81	25.38	26.40	25.44	24.66	31.04
129	33.45	35.18	40.46	34.65	28.44	33.12	31.98	26.46
132	33.68	34.11	38.40	27.27	42.41	33.24	31.32	28.79
136	25.80	28.34	59.49	30.74	29.66	28.47	29.66	37.05
137	50.09	54.56	51.71	44.00	47.66	43.55	34.34	38.72
140	44.34	39.69	40.19	40.70	44.22	53.82	40.89	44.34
143	28.95	38.00	39.90	41.70	37.10	32.79	27.30	40.89
146	35.54	43.70	39.74	41.16	34.44	34.83	36.87	40.32
150	46.95	43.62	54.65	77.43	45.45	48.41	50.76	47.28
151	26.90	19.48	34.73	14.85	23.33	23.93	26.10	26.43
155	24.23	21.53	24.53	21.40	22.55	21.88	24.73	22.28
158	22.98	29.40	35.68	25.03	23.35	24.05	29.30	23.60
162	49.85	49.56	39.54	45.57	35.90	42.36	36.77	54.69
166	41.58	36.77	40.28	35.82	38.63	41.15	39.14	43.05
Mean	**31.93**	**33.24**	**37.16**	**33.61**	**31.66**	**32.86**	**30.08**	**34.03**

8IP (pg/ml) Raw Data – HHT (n=22)

Random	TT1		TT2		TT3		TT4	
Number	pre	end	pre	end	pre	end	pre	end
103	13.18	7.50	9.80	15.25	11.00	12.03	8.05	11.53
105	38.18	22.55	24.23	28.15	19.55	25.75	24.78	27.35
109	29.85	30.50	31.95	22.23	23.68	29.85	32.15	26.20
112	15.20	26.80	25.20	29.78	24.78	27.09	32.79	51.90
116	21.18	21.55	30.88	26.33	20.35	23.33	24.55	29.70
118	28.85	29.13	22.85	28.02	26.70	25.83	27.23	27.75
122	35.07	27.66	32.60	30.74	30.57	31.77	47.13	43.08
123	37.32	34.38	40.10	41.96	46.04	46.62	41.84	27.54
128	28.29	27.93	26.79	25.08	27.66	31.04	23.78	29.37
130	48.23	38.19	34.13	52.37	29.85	38.99	36.27	48.65
131	45.05	56.03	43.61	43.20	29.03	39.45	33.39	56.60
133	38.12	38.33	39.96	35.91	34.04	42.71	39.14	46.44
138	39.44	41.33	38.39	44.18	40.43	41.33	38.18	42.96
142	38.73	32.58	32.25	30.78	30.60	45.45	27.87	48.41
144	31.74	29.76	28.05	43.19	35.87	39.32	38.55	35.52
145	47.21	44.01	40.71	37.73	38.24	35.79	38.24	46.77
152	56.93	43.08	43.65	39.43	35.18	48.63	47.13	39.25
154	24.85	---	22.50	35.30	31.65	20.90	12.90	24.93
156	25.63	24.65	35.75	25.93	24.15	26.03	19.20	24.60
159	27.80	26.39	24.53	32.88	27.23	23.60	24.63	20.28
167	32.54	39.21	37.35	40.44	40.65	38.61	38.31	38.42
172	34.62	39.11	35.60	44.24	37.92	31.76	40.13	41.30
Mean	**33.54**	**32.41**	**31.86**	**34.23**	**30.23**	**32.99**	**31.65**	**35.84**

TNFα

TNFα (pg/ml) Raw Data – PLA (n=19)

Random	TT1		TT2		TT3		TT4	
Number	pre	end	pre	end	pre	end	pre	end
102	4.80	5.57	44.75	54.30	21.38	31.56	21.07	29.41
106	3.21	3.81	3.37	4.18	3.64	5.17	4.17	5.18
107	5.92	5.14	4.45	5.21	5.11	6.87	5.35	4.58
110	5.45	5.11	9.73	8.84	10.58	14.38	12.59	16.77
113	1.81	1.89	1.85	3.01	2.14	2.11	3.09	2.36
117	8.00	10.20	6.46	8.10	8.15	8.81	6.23	8.23
120	6.31	6.96	7.35	8.48	4.31	6.05	4.75	4.89
125	10.53	12.49	10.15	12.35	10.27	11.55	12.70	12.17
126	6.03	7.11	5.57	7.32	7.80	8.44	6.21	8.23
139	4.51	5.18	3.85	5.29	4.45	5.81	4.65	4.53
141	3.40	4.25	2.71	4.28	2.90	4.00	2.88	4.13
147	6.32	7.90	---	---	5.56	7.41	5.33	7.73
148	2.05	2.51	1.82	1.92	1.33	1.97	1.72	1.65
149	5.66	6.97	6.37	8.92	5.50	8.17	6.13	8.62
153	12.93	27.13	7.70	27.64	23.24	16.13	10.53	23.52
157	3.66	4.51	4.83	4.86	3.32	4.20	3.29	3.92
160	7.14	9.74	6.63	7.14	6.74	10.52	6.92	8.55
161	5.76	6.87	5.74	6.86	5.84	6.67	6.02	6.41
171	2.72	3.70	3.01	3.76	2.31	3.77	2.52	3.57
Mean	**5.59**	**7.21**	**7.57**	**10.14**	**7.08**	**8.61**	**6.64**	**8.66**

TNFα (pg/ml) Raw Data – LHT (n=20)

Random Number	TT1		TT2		TT3		TT4	
	pre	end	pre	end	pre	end	pre	end
101	6.94	6.38	5.36	5.91	5.24	6.88	5.36	6.37
104	7.40	6.72	7.76	8.68	7.74	8.33	8.24	7.51
108	8.05	10.84	7.07	9.81	12.39	12.92	9.75	---
111	4.56	5.55	4.07	4.24	3.31	4.86	3.40	4.78
114	9.12	12.61	6.87	9.58	7.78	9.11	6.26	8.35
115	5.57	9.37	8.01	12.58	6.74	10.45	6.83	10.02
127	4.00	4.97	4.66	4.74	4.89	5.41	4.93	7.83
129	5.47	6.85	4.22	5.26	5.81	6.03	4.71	5.38
132	4.48	5.92	3.45	4.00	4.92	4.69	4.58	5.15
136	8.17	7.39	6.33	7.99	6.08	7.68	8.16	8.52
137	7.34	8.31	9.56	11.97	7.58	9.40	6.93	11.26
140	4.66	5.19	4.48	4.85	3.24	4.16	3.42	4.06
143	5.77	8.06	5.05	6.58	4.28	5.61	4.01	5.26
146	9.42	10.28	6.26	8.73	7.19	8.79	5.91	8.24
150	8.10	8.08	8.33	11.27	13.24	12.04	9.93	12.49
151	7.72	7.69	7.20	6.77	6.05	7.47	6.02	6.69
155	8.62	10.75	6.85	6.94	7.35	6.15	6.29	6.24
158	9.81	11.90	8.52	13.59	8.75	12.62	7.86	12.46
162	4.87	5.59	4.18	6.07	4.78	5.88	4.63	5.37
166	3.67	4.18	2.43	2.97	2.93	3.10	2.09	2.83
Mean	**6.69**	**7.83**	**6.03**	**7.63**	**6.51**	**7.58**	**5.97**	**7.31**

TNFα (pg/ml) Raw Data – HHT (n=22)

Random	TT1		TT2		TT3		TT4	
Number	pre	end	pre	end	pre	end	pre	end
103	7.97	7.48	5.22	5.59	6.79	7.51	5.58	6.64
105	4.98	6.59	5.64	7.05	4.66	6.92	6.02	7.18
109	5.84	5.90	5.53	7.32	6.34	7.83	5.92	8.94
112	4.92	4.53	3.17	3.93	3.33	3.97	3.42	4.56
116	6.17	6.01	5.75	7.11	4.55	8.75	7.03	8.08
118	7.03	9.99	5.00	5.89	4.56	5.92	5.18	6.36
122	7.19	9.05	6.10	8.57	5.37	7.02	6.25	7.42
123	4.06	5.63	2.57	4.27	3.33	3.91	4.07	4.18
128	8.30	11.12	9.01	11.20	6.90	9.90	7.26	11.07
130	6.49	7.78	5.60	6.78	7.12	8.32	6.47	7.51
131	4.80	9.44	4.18	6.66	4.96	6.80	5.65	4.39
133	2.36	2.45	1.78	2.12	1.87	2.09	1.95	1.76
138	4.31	6.35	3.12	4.60	3.17	3.74	3.02	3.56
142	5.21	5.60	4.83	6.23	4.92	6.03	7.46	8.08
144	4.42	7.45	5.89	7.97	4.67	6.55	4.82	7.52
145	5.54	9.53	5.62	7.59	5.73	6.21	5.30	6.44
152	4.54	5.08	4.51	6.12	3.87	6.13	4.20	5.76
154	2.99	---	3.39	3.54	4.49	7.09	4.82	6.04
156	5.13	5.50	4.62	4.29	3.63	4.49	4.18	4.14
159	13.26	15.85	14.10	18.14	16.05	15.52	15.91	17.61
167	5.93	6.36	5.42	6.73	3.63	4.57	3.87	4.39
172	9.05	9.42	7.12	8.93	7.08	7.39	5.97	5.94
Mean	**5.93**	**7.48**	**5.37**	**6.85**	**5.32**	**6.67**	**5.65**	**6.71**

IL-6

IL-6 (pg/ml) Raw Data – PLA (n=19)

Random Number	TT1		TT2		TT3		TT4	
	pre	end	pre	end	pre	end	pre	end
102	1.52	4.49	70.79	103.00	32.85	55.30	30.77	40.95
106	5.62	7.40	4.86	6.84	5.59	7.70	4.90	7.44
107	4.08	3.68	4.11	5.51	5.19	7.67	5.20	4.88
110	0.20	0.97	8.88	6.75	15.54	23.94	13.49	24.41
113	2.58	7.94	2.42	5.16	1.77	6.71	3.45	6.13
117	2.67	11.03	1.94	6.58	1.84	5.81	1.41	6.26
120	0.23	2.73	0.77	2.70	0.23	1.44	0.34	1.22
125	0.62	2.56	0.94	2.91	0.67	2.13	0.93	2.24
126	0.92	3.91	0.66	3.10	1.39	3.80	0.96	3.46
139	2.02	4.06	2.02	5.80	1.89	4.67	1.62	2.42
141	7.89	8.42	4.58	4.99	4.88	5.14	3.69	5.16
147	1.79	4.65	---	---	1.67	5.52	1.43	4.53
148	0.27	2.49	0.62	1.05	0.27	1.68	0.62	1.78
149	0.25	2.89	2.01	5.53	0.19	2.10	0.53	1.77
153	13.66	34.33	6.44	28.95	24.02	20.14	10.45	22.66
157	0.37	2.06	0.59	2.37	2.50	3.68	0.68	1.84
160	2.02	4.30	2.05	5.02	1.94	15.43	2.36	6.00
161	8.56	13.07	11.37	9.86	14.72	11.42	10.38	10.87
171	1.92	4.70	3.85	6.79	2.05	10.88	0.70	4.69
Mean	**3.01**	**6.61**	**7.16**	**11.83**	**6.27**	**10.27**	**4.94**	**8.35**

IL-6 (pg/ml) Raw Data – LHT (n=20)

Random Number	TT1		TT2		TT3		TT4	
	pre	end	pre	end	pre	end	pre	end
101	0.56	0.97	0.76	1.12	0.81	2.05	0.56	1.62
104	0.31	0.96	0.49	2.28	0.00	1.21	0.51	0.68
108	11.21	10.40	12.70	9.51	8.61	8.65	4.17	---
111	1.00	2.22	1.17	4.53	0.48	10.73	0.47	3.33
114	0.39	4.47	0.15	4.95	0.27	1.89	0.42	2.49
115	0.55	4.47	0.78	6.42	0.49	5.85	0.36	1.38
127	5.33	7.09	5.93	7.26	5.77	6.45	6.18	10.71
129	0.69	1.44	0.39	1.86	2.15	2.69	0.42	1.15
132	7.54	11.72	6.06	8.74	9.86	10.43	6.85	11.64
136	46.32	43.49	39.07	41.52	38.38	42.21	43.91	42.01
137	1.45	2.82	1.28	3.20	0.94	3.00	1.01	3.46
140	0.64	2.98	1.88	4.03	0.99	4.17	1.34	3.92
143	0.64	1.64	0.47	1.86	0.65	1.63	0.77	2.00
146	15.20	16.87	10.47	17.87	20.64	30.58	20.32	29.38
150	4.19	5.23	5.54	7.52	11.62	13.00	8.68	10.17
151	1.66	5.37	2.39	5.48	0.63	3.31	2.36	3.84
155	0.93	4.16	0.26	2.86	0.67	1.95	0.49	1.99
158	0.59	2.22	0.07	2.04	0.10	1.60	0.02	1.32
162	0.80	4.86	0.71	4.25	0.71	3.90	1.02	2.62
166	1.77	5.37	2.87	3.62	5.43	8.58	3.60	6.93
Mean	**5.09**	**6.94**	**4.67**	**7.05**	**5.46**	**8.19**	**5.17**	**7.40**

IL-6 (pg/ml) Raw Data – HHT (n=22)

Random Number	TT1		TT2		TT3		TT4	
	pre	end	pre	end	pre	end	pre	end
103	1.91	2.61	1.86	3.09	2.43	5.21	1.98	3.49
105	0.45	3.60	0.81	2.49	0.39	3.05	0.82	1.94
109	1.15	3.11	0.93	4.31	1.91	5.46	1.52	4.57
112	33.12	36.78	18.72	23.83	14.76	18.64	15.56	16.50
116	2.97	7.42	2.28	9.87	1.66	15.82	2.55	7.33
118	1.23	4.92	0.99	4.84	1.06	4.52	2.13	5.41
122	1.01	3.11	0.76	2.99	0.80	2.36	1.19	3.08
123	2.41	5.39	2.02	3.72	2.01	3.75	3.98	5.26
128	1.82	5.61	1.93	7.06	2.30	5.07	2.30	6.91
130	2.40	5.58	2.34	4.24	2.40	4.02	2.53	3.36
131	6.55	13.97	7.17	11.64	7.16	13.35	6.21	5.05
133	30.29	27.50	13.79	17.54	10.10	15.59	14.06	11.57
138	3.19	5.86	2.84	3.96	2.10	3.82	2.62	4.39
142	0.29	2.38	0.40	2.56	0.51	2.73	0.87	1.58
144	1.26	2.63	1.26	4.64	1.73	3.91	1.56	3.14
145	1.14	9.61	1.62	5.87	0.85	6.45	0.63	5.40
152	9.62	9.33	9.26	10.63	9.94	13.37	10.69	13.75
154	0.51	---	0.49	4.27	0.50	5.38	0.92	3.32
156	6.22	7.06	5.42	7.29	3.31	7.36	3.63	6.38
159	21.73	24.73	23.04	30.37	25.11	27.33	30.59	35.96
167	10.04	13.63	7.98	10.40	8.72	10.06	6.28	11.33
172	2.55	4.36	1.61	3.47	2.32	5.21	3.98	7.47
Mean	**6.45**	**9.49**	**4.89**	**8.14**	**4.64**	**8.29**	**5.30**	**7.60**

IL-10

IL-10 (pg/ml) Raw Data – PLA (n=19)

Random Number	TT1		TT2		TT3		TT4	
	pre	end	pre	end	pre	end	pre	end
102	9.56	25.46	341.00	829.00	139.00	340.00	162.00	239.00
106	26.99	28.77	27.90	31.34	26.07	37.55	22.58	33.69
107	43.05	39.29	50.49	40.86	54.99	60.37	60.01	45.83
110	7.27	5.48	62.45	59.33	112.00	124.00	93.12	127.00
113	6.77	11.64	7.38	7.38	6.42	7.78	6.24	8.76
117	14.19	18.06	16.30	14.94	10.96	16.90	15.14	19.85
120	7.40	9.14	8.57	8.15	8.05	8.81	8.81	8.15
125	11.20	14.46	13.38	12.72	15.32	12.68	13.64	13.12
126	4.38	8.86	3.20	13.94	4.78	6.73	4.87	9.44
139	12.39	16.37	11.81	20.48	14.16	21.88	16.30	15.97
141	239.00	288.00	119.00	146.00	114.00	140.00	120.00	132.00
147	40.80	36.31	---	---	25.46	29.46	27.25	27.60
148	5.82	13.36	10.15	11.86	5.86	8.43	7.26	6.94
149	1.25	6.54	0.90	4.18	0.65	3.56	0.38	0.50
153	23.24	63.33	9.70	80.43	36.70	27.76	18.02	36.70
157	9.57	12.64	8.36	8.91	9.90	9.28	6.43	9.35
160	21.31	24.66	18.09	19.66	22.10	65.05	26.06	29.90
161	23.81	31.44	31.44	31.98	17.16	18.68	13.03	14.80
171	19.30	30.67	26.69	31.27	15.04	19.78	14.66	17.30
Mean	**27.75**	**36.03**	**42.60**	**76.25**	**33.61**	**50.46**	**33.46**	**41.89**

IL-10 (pg/ml) Raw Data – LHT (n=20)

Random Number	TT1		TT2		TT3		TT4	
	pre	end	pre	end	pre	end	pre	end
101	10.96	10.01	6.41	7.47	10.92	13.49	11.41	12.49
104	14.66	10.59	18.53	18.61	13.60	12.56	14.88	9.70
108	20.04	17.59	18.89	29.42	112.00	194.00	31.10	
111	12.05	8.14	13.45	11.78	8.90	18.61	8.27	11.41
114	17.41	35.95	14.89	32.17	14.29	15.09	14.79	33.92
115	8.00	27.56	12.07	21.84	6.38	20.80	7.61	5.71
127	13.01	9.19	12.92	10.18	12.79	14.17	15.99	20.84
129	0.35	0.96	0.11	1.26	6.24	4.01	0.35	0.27
132	39.41	51.91	33.44	34.13	48.01	45.18	40.93	50.95
136	331.00	297.00	362.00	409.00	292.00	315.00	372.00	368.00
137	5.16	10.86	7.60	7.41	17.20	16.96	13.50	13.02
140	17.32	15.56	16.27	12.53	14.06	14.84	13.27	12.96
143	1.79	3.67	2.44	2.22	1.37	2.05	2.14	2.14
146	146.00	150.00	62.15	83.06	82.83	98.21	78.27	105.00
150	3.46	5.27	4.23	6.12	6.49	6.96	5.23	5.37
151	4.75	8.84	11.71	8.17	2.54	4.88	3.97	5.89
155	19.26	40.64	10.19	15.80	14.03	14.62	15.21	15.55
158	12.05	10.11	6.61	10.94	6.57	10.19	7.51	12.50
162	8.96	14.61	6.37	11.41	5.57	11.73	6.59	7.95
166	11.51	12.80	8.80	7.55	12.85	18.86	11.62	19.54
Mean	**34.86**	**37.06**	**31.45**	**37.05**	**34.43**	**42.61**	**33.73**	**37.54**

IL-10 (pg/ml) Raw Data – HHT (n=22)

Random Number	TT1		TT2		TT3		TT4	
	pre	end	pre	end	pre	end	pre	end
103	3.91	5.67	3.99	5.92	5.71	5.27	6.28	3.43
105	10.30	24.95	8.11	14.67	6.21	16.67	8.36	13.48
109	2.60	3.51	3.67	3.21	5.92	7.66	4.59	6.59
112	423.00	325.00	56.05	70.64	82.58	95.89	84.39	85.06
116	9.83	31.93	11.17	21.22	5.98	20.83	10.55	17.30
118	10.03	16.15	10.52	13.53	9.50	14.03	11.48	11.43
122	3.02	5.06	3.32	8.96	2.78	4.53	3.71	5.97
123	63.04	84.98	29.65	37.89	33.69	42.14	54.52	57.66
128	14.68	22.43	19.27	22.19	15.19	17.64	14.13	30.23
130	27.64	45.88	18.25	20.79	34.82	27.42	29.98	40.21
131	47.47	87.02	45.29	56.63	45.66	71.73	45.00	38.78
133	783.00	602.00	301.00	339.00	233.00	345.00	306.00	238.00
138	13.33	19.72	9.85	16.30	11.70	13.69	19.44	21.27
142	4.40	6.90	8.83	9.93	7.82	8.74	8.93	6.95
144	16.07	19.40	15.90	21.66	17.29	18.68	17.64	24.90
145	8.35	28.48	8.75	17.41	6.45	14.03	8.80	11.88
152	11.08	8.84	8.21	10.37	9.20	10.05	8.21	9.16
154	4.71	---	5.09	5.48	37.03	29.46	43.11	43.24
156	17.32	20.51	18.15	24.02	13.04	28.25	16.77	17.35
159	72.66	90.08	84.48	121.00	87.93	102.00	117.00	167.00
167	43.50	41.12	65.43	71.66	25.30	29.66	27.39	31.29
172	15.81	17.34	6.80	15.81	12.06	14.42	9.71	19.73
Mean	**72.99**	**71.76**	**33.72**	**42.20**	**32.22**	**42.63**	**38.91**	**40.95**

IL-1β

IL-1β (pg/ml) Raw Data – PLA (n=19)

Random Number	TT1		TT2		TT3		TT4	
	pre	end	pre	end	pre	end	pre	end
102	0.02	0.04	32.31	36.12	14.65	18.33	13.64	19.48
106	1.02	0.83	1.03	0.89	0.96	1.12	0.92	1.32
107	0.61	0.29	0.59	0.28	1.41	1.52	0.93	0.53
110	0.09	0.07	5.52	3.72	6.37	8.31	6.24	9.50
113	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00
117	0.32	0.53	0.20	0.15	0.26	0.29	0.14	0.15
120	0.15	0.14	0.18	0.16	0.20	0.22	0.16	0.18
125	0.10	0.13	0.05	0.08	0.08	0.10	0.17	0.08
126	0.39	0.38	0.37	0.48	0.88	0.58	0.17	0.30
139	0.72	0.55	0.60	0.68	0.62	0.69	0.49	0.49
141	3.34	2.47	1.24	1.61	1.33	0.92	0.98	1.30
147	0.00	0.01	---	---	0.02	0.01	0.01	0.01
148	0.06	0.02	0.15	0.10	0.07	0.06	0.07	0.07
149	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
153	0.28	3.08	0.06	3.19	1.83	0.54	0.27	1.31
157	0.21	0.22	0.22	0.23	0.21	0.21	0.13	0.17
160	0.07	0.11	0.12	0.11	0.09	0.13	0.09	0.09
161	0.20	0.68	0.39	0.58	0.45	0.33	0.23	0.26
171	0.06	0.12	0.03	0.03	0.01	0.05	0.02	0.03
Mean	**0.40**	**0.51**	**2.39**	**2.69**	**1.55**	**1.76**	**1.30**	**1.86**

IL-1β (pg/ml) Raw Data – LHT (n=20)

Random Number	TT1		TT2		TT3		TT4	
	pre	end	pre	end	pre	end	pre	end
101	0.24	0.21	0.24	0.09	0.17	0.22	0.19	0.24
104	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
108	0.05	0.01	0.05	0.10	0.01	0.03	0.01	---
111	0.00	0.00	0.03	0.03	0.02	0.05	0.02	0.03
114	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
115	0.00	0.01	0.02	0.03	0.01	0.01	0.00	0.01
127	1.47	1.69	2.14	1.49	1.83	1.24	1.41	3.44
129	0.16	0.23	0.15	0.19	0.25	0.14	0.13	0.14
132	0.38	0.37	1.16	1.18	2.02	2.19	0.44	1.50
136	0.27	0.31	0.31	0.47	0.25	0.19	0.31	0.36
137	0.01	0.04	0.02	0.03	0.01	0.00	0.00	0.01
140	0.00	0.05	0.04	0.06	0.08	0.05	0.02	0.07
143	0.01	0.02	0.03	0.02	0.03	0.04	0.04	0.08
146	0.15	0.16	0.01	0.12	0.25	0.40	0.22	0.36
150	1.15	0.76	1.92	3.03	5.22	4.07	3.03	3.90
151	0.13	0.06	0.15	0.02	0.02	0.03	0.07	0.02
155	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
158	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
162	0.49	0.50	0.17	0.76	0.40	0.71	0.48	0.48
166	0.10	0.02	0.07	0.10	0.27	0.50	0.06	0.55
Mean	**0.23**	**0.22**	**0.33**	**0.39**	**0.54**	**0.49**	**0.32**	**0.59**

IL-1β (pg/ml) Raw Data – HHT (n=22)

Random Number	TT1		TT2		TT3		TT4	
	pre	end	pre	end	pre	end	pre	end
103	0.57	0.57	0.56	0.53	0.53	0.54	0.43	0.38
105	0.08	0.16	0.12	0.19	0.11	0.06	0.11	0.14
109	0.20	0.26	0.19	0.19	0.29	0.26	0.14	0.22
112	0.13	0.18	0.70	0.66	0.54	0.63	0.65	0.45
116	0.08	0.00	0.08	0.03	0.06	0.03	0.03	0.06
118	0.01	0.00	0.04	0.10	0.08	0.19	0.15	0.54
122	0.01	0.01	0.01	0.06	0.02	0.02	0.01	0.06
123	0.06	0.13	0.41	0.82	0.66	0.79	1.22	1.04
128	0.34	1.05	0.32	0.35	0.14	0.37	0.26	1.43
130	0.23	1.31	0.16	0.00	0.05	0.01	0.03	0.11
131	2.26	4.51	1.60	2.65	2.50	3.26	2.50	1.07
133	0.17	0.06	0.53	0.64	0.57	0.54	0.52	0.41
138	0.32	0.52	0.28	0.22	0.29	0.27	0.26	0.56
142	0.01	0.01	0.17	0.10	0.10	0.02	0.03	0.01
144	0.01	0.03	0.04	0.00	0.03	0.03	0.02	0.00
145	0.01	0.09	0.05	0.03	0.01	0.03	0.01	0.03
152	0.41	0.34	0.23	0.52	0.15	0.30	0.21	0.31
154	0.02	---	0.02	0.01	0.01	0.05	0.01	0.03
156	0.13	0.19	0.06	0.09	0.09	0.27	0.11	0.08
159	3.04	4.36	3.10	5.72	4.62	4.82	4.65	5.03
167	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
172	4.09	3.05	0.23	0.00	3.58	1.89	2.06	2.01
Mean	**0.55**	**0.80**	**0.40**	**0.59**	**0.66**	**0.65**	**0.61**	**0.64**

IL-1ra

IL-1ra (pg/ml) Raw Data – PLA (n=19)

Random Number	TT1		TT2		TT3		TT4	
	pre	end	pre	end	pre	end	pre	end
102	0.00	0.00	107.00	173.00	81.13	96.29	84.72	103.00
106	38.63	43.89	42.31	50.22	50.22	50.22	40.73	63.94
107	45.11	47.00	46.37	58.66	61.05	70.43	62.83	68.69
110	78.37	71.23	66.43	88.37	77.78	73.02	76.59	75.41
113	2.19	4.96	0.31	3.30	0.16	2.19	0.97	3.30
117	1.79	6.56	3.21	2.83	2.47	0.44	2.47	8.88
120	42.30	45.46	29.40	30.05	22.83	35.25	35.89	23.49
125	2.87	5.81	3.54	7.91	5.02	5.41	5.41	2.87
126	0.95	1.95	0.74	2.24	0.56	3.90	0.40	1.95
139	22.10	25.87	31.88	37.08	26.63	47.28	34.86	28.13
141	5.75	14.76	10.58	21.90	10.58	9.57	20.23	15.30
147	0.00	0.01	---	---	0.00	0.54	0.00	11.75
148	6.11	7.93	3.83	10.43	3.83	6.71	2.77	11.07
149	0.00	0.37	0.00	0.09	0.00	0.16	0.00	0.65
153	1.86	4.53	4.96	4.96	1.24	6.78	1.24	5.85
157	17.66	10.97	8.51	16.61	6.18	14.01	6.63	9.48
160	41.59	59.57	20.42	34.84	36.28	42.11	27.29	46.92
161	187.00	170.00	187.00	180.00	203.00	207.00	183.00	166.00
171	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Mean	**26.01**	**27.41**	**31.47**	**40.14**	**31.00**	**35.33**	**30.84**	**34.04**

IL-1ra (pg/ml) Raw Data – LHT (n=20)

Random Number	TT1		TT2		TT3		TT4	
	pre	end	pre	end	pre	end	pre	end
101	0.00	0.00	0.00	0.00	0.00	21.39	0.00	0.00
104	1.65	1.65	0.68	4.33	0.32	1.94	0.19	0.09
108	9.74	0.66	7.72	3.27	2.69	4.49	1.60	---
111	0.50	0.72	0.50	0.50	3.30	1.24	0.00	1.24
114	0.00	4.82	0.00	0.66	0.00	2.47	0.00	0.00
115	0.11	1.18	0.00	2.47	0.00	0.66	0.00	2.47
127	16.40	40.12	17.13	22.22	48.43	40.82	35.18	26.57
129	13.63	15.55	20.01	25.13	24.62	26.69	27.74	29.31
132	63.66	68.52	49.68	37.57	49.68	48.47	50.29	46.04
136	35.10	34.55	32.90	28.57	26.44	28.03	25.38	26.44
137	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
140	4.71	6.84	5.23	6.30	4.71	10.28	1.93	6.84
143	3.54	2.89	1.72	4.97	1.72	2.89	1.72	4.97
146	9.71	15.00	11.58	18.56	8.35	13.51	10.17	12.05
150	2.26	4.62	1.71	1.98	5.81	7.99	6.23	5.81
151	1.30	3.20	1.00	0.12	0.00	0.00	0.12	0.48
155	3.60	7.60	3.96	3.60	5.11	5.51	5.11	4.33
158	0.74	1.47	0.05	2.66	0.35	2.66	0.16	0.59
162	34.98	55.68	37.86	52.24	38.43	40.16	35.56	37.86
166	4.71	9.69	11.47	9.69	4.71	10.87	2.35	3.24
Mean	**10.32**	**13.74**	**10.16**	**11.24**	**11.23**	**13.50**	**10.19**	**10.96**

IL-1ra (pg/ml) Raw Data – HHT (n=22)

Random Number	TT1		TT2		TT3		TT4	
	pre	end	pre	end	pre	end	pre	end
103	112.00	114.00	93.21	93.74	100.00	93.74	104.00	81.45
105	19.78	22.44	16.09	27.81	15.05	20.84	15.57	24.58
109	24.13	31.52	18.66	15.22	11.78	30.19	12.46	36.78
112	5.19	5.19	10.83	17.52	8.49	15.67	10.83	9.07
116	1.00	2.00	1.30	3.20	0.72	2.78	0.02	1.64
118	0.83	2.55	3.19	4.25	2.86	6.66	1.98	7.99
122	0.00	0.00	0.00	0.37	0.00	0.00	0.00	0.00
123	0.06	6.71	30.05	36.54	34.60	42.30	31.35	42.93
128	7.19	13.51	3.35	16.40	2.77	22.95	12.08	28.02
130	0.00	0.72	0.00	0.00	0.00	0.00	0.00	0.00
131	4.83	10.57	18.73	8.55	10.05	6.16	7.10	12.67
133	0.04	0.65	18.31	20.58	17.56	19.83	22.85	16.80
138	5.86	5.19	7.95	12.31	2.11	8.66	4.53	3.27
142	7.73	7.32	10.30	6.11	3.54	6.11	5.72	8.99
144	26.60	23.59	32.18	32.69	18.67	23.59	19.65	32.18
145	17.60	21.59	10.05	17.60	16.49	16.49	11.09	14.83
152	1.64	3.20	0.12	1.30	0.00	2.38	0.00	0.72
154	4.16	---	5.19	8.49	7.36	15.05	10.24	7.92
156	5.33	7.12	4.11	8.04	4.44	6.18	5.73	6.75
159	121.00	149.00	110.00	132.00	145.00	153.00	155.00	135.00
167	0.32	5.23	1.54	1.93	0.00	0.32	0.00	0.00
172	0.00	0.00	0.00	0.00	0.00	0.82	0.44	0.00
Mean	**16.60**	**20.58**	**17.96**	**21.12**	**18.25**	**22.44**	**19.57**	**21.44**

COMPREHENSIVE BIBLIOGRAPHY

Acin, S., Navarro, M. A., Arbones-Mainar, J. M., Guillen, N., Sarria, A. J., Carnicer, R., et al. (2006). Hydroxytyrosol Administration Enhances Atherosclerotic Lesion Development in Apo E Deficient Mice. *J. Biochem.*, 383–391.

Alessio, H. M., Hagerman, A. E., Fulkerson, B. K., Ambrose, J., Rice, R. E., & Wiley, R. L. (2000). Generation of reactive oxygen species after exhaustive aerobic and isometric exercise. *Medicine & Science in Sports & Exercise*, 1576-1581.

Aruoma, O. I., Deiana, M., Jenner, A., Halliwell, B., Kaur, H., Banni, S., et al. (1998). Effect of Hydroxytyrosol Found in Extra Virgin Olive Oil on Oxidative DNA Damage and on Low-Density Lipoprotein Oxidation. *Journal of Agricultural and Food Chemistry*, 5181-5187.

Aviram, M., & Fuhrman, B. (1998). LDL oxidation by arterial wall macrophages depends on the oxidative status in the lipoprotein and in the cells: Role of prooxidants vs. antioxidants. *Molecular and Cellular Biochemistry*, 149–159.

Baldioli, M., Servili, M., Perretti, G., & Montedoro, G. F. (1996). Antioxidant Activity of Tocopherols and Phenolic Compounds of Virgin Olive Oil. *Journal of the American Oil Chemists' Society*, 1589-1593.

Baydur, A., Alavy, B., Nawathe, A., Liu, S., Louie, S., & Sharma, O. (2010). Fatigue and plasma cytokine concentrations at rest and during exercise in patients with sarcoidosis. *The Clinical Respiratory Journal*, 1-9.

Berzosa, C., Cebrian, I., Fuentes-Broto, L., Gomez-Trullen, E., Piedrafita, E., Martınez-Balların, E., et al. (2011). Acute Exercise Increases Plasma Total Antioxidant Status and Antioxidant Enzyme Activities in Untrained Men. *Journal of Biomedicine and Biotechnology*, 1-7.

Bigelman, K. A., Fan, E. H., Chapman, D. P., Freese, E. C., Trilk, J. L., & Cureton, K. J. (2010). Effects of Six Weeks of Quercetin Supplementation on Physical Performance in ROTC Cadets. *Military Medicine*, 791-798.

Bogani, P., Gallie, C., Villa, M., & Visioli, F. (2007). Postprandial anti-inflammatory and antioxidant effects of extra virgin olive oil. *Atheroslerosis* (190), 181-186.

Carluccio, M. A., Siculella, L., Ancora, M. A., Massaro, M., Scoditti, E., Storelli, C., et al. (2003). Olive Oil and Red Wine Antioxidant Polyphenols Inhibit Endothelial Activation. *Arteriosclerosis, Thrombosis, and Vascular Biology, Journal of the American Heart Association*, 622-629.

Carrasco-Pancorbo, A., Cerretani, L., Bendini, A., Segura-Carretero, A., Del Carlo, M., Gallina-Toschi, T., et al. (2005). Evaluation of the Antioxidant Capacity of Individual Phenolic Compounds in Virgin Olive Oil. *Journal of Agricultural and Food Chemistry*, 8918-8925.

Cerami, A. (1992). Inflammatory Cytokines. *Clinical Immunology and Immunopathology*, S3-S10.

Cesari, M., Pahor, M., Bartali, B., Cherubini, A., Penninx, B. W., Williams, G. R., et al. (2004). Antioxidants and physical performance in elderly persons: the Invecchiare in Chianti (InCHIANTI) study. *The American Journal of Clinical Nutrition*, 289-294.

Cicerale, S., Lucas, L., & Keast, R. (2010). Biological Activities of Phenolic Compounds Present in Virgin Olive Oil. *International Journal of Molecular Sciences*, 458-479.

Coni, E., Di Benedetto, R., Di Pasquale, M., Masella, R., Modesti, D., Mattei, R., et al. (2000). Protective Effect of Oleuropein, an Olive Oil Biophenol, on Low Density Lipoprotein Oxidizability in Rabbits . *Lipids*, 45-54.

Cornwell, D. G., & Ma, J. (2008). Nutritional Benefit of Olive Oil: The Biological Effects of Hydroxytyrosol and Its Arylating Quinone Adducts. *Journal of Agriculture and Food Chemistry*, 8774–8786.

Covas, M.-I. (2007). Olive oil and the cardiovascular system. *Pharmacological Research*, 175–186.

Covas, M.-I., de la Torre, K., Farre-Albaladejo, M., Kaikkonen, J., Fito, M., Lopez-Sabater, C., et al. (2006). Postprandial LDL phenolic content and LDL oxidation are modulated by olive oil phenolic compounds in humans. *Free Radical Biology & Medicine*, 608-616.

Cureton, K. J., Tomporowski, P. D., Singhal, A., Pasley, J. D., Bigelman, K. A., Lambourne, K., et al. (2009). Dietary quercetin supplementation is not ergogenic in untrained men. *Journal of Applied Physiology*, 1095–1104.

Davis, J. M., Carlstedt, C. J., Chen, S., Carmichael, M. D., & Murphy, E. A. (2010). The Dietary Flavonoid Quercetin Increases VO2max and Endurance Capacity. *International Journal of Sport Nutrition and Exercise Metabolism* (20), 56-62.

Davis, J. M., Murphy, E. A., Carmichael, M. D., & Davis, B. (2009). Quercetin increases brain and muscle mitochondrial biogenesis and exercise tolerance. *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology*, R1071–R1077.

Deiana, M., Aruoma, O. I., Bianchi, M. D., Spencer, J. P., Kaur, H., Halliwell, B., et al. (1999). Inhibition of peroxynitrite dependent DNA base modification and tyrosine nitration by the extra virgin olive oil-derived antioxidant hydroxytyrosol. *Free Radical Biology & Medicine*, 762-769.

D'Angelo, S., Manna, C., Migliardi, V., Mazzoni, O., Morrica, P., Capasso, G., et al. (2001). Pharmacokinetics and metabolism of hydroxytyrosol, a natural antioxidant from olive oil. *Drug Metabolism and Disposition*, 1492–1498.

Du Clos, T. W., & Mold, C. (2004). C-Reactive Protein. *Immunologic Research* (30/3), 261-277.

EFSA Panel on Dietetic Products, Nutrition and Allergies. (2011). Scientific Opinion on the substantiation of health claims related to polyphenols in olive and protection of LDL particles from oxidative damage (ID 1333, 1638, 1639, 1696, 2865), maintenance of normal blood HDL-cholesterol concentrations (ID 1639), maintenance of normal blood pressure (ID 3781), "anti-inflammatory properties" (ID 1882), "contributes to the upper respiratory tract health" (ID 3468), "can help to maintain a normal function of gastrointestinal tract" (3779), and "contributes to body defences against external agents" (ID 3467) pursuant to Article 13(1) of Regulation (EC) No 1924/20061. *EFSA Journal* (pp. 1-25). Parma, Italy: European Food Safety Authority.

Egert, S., Wolffram, S., Bosy-Westphal, A., Boesch-Saadatmandi, C., Wagner, A. E., Frank, J., et al. (2008). Daily Quercetin Supplementation Dose-Dependently Increases Plasma Quercetin Concentrations in Healthy Humans. *The Journal of Nutrition*, 1615-1621.

Faine, L. A., Rodrigues, H. G., Galhardi, C. M., Ebaid, G. M., Diniz, Y. S., Padovani, C. R., et al. (2006). Effects of olive oil and its minor constituents on serum lipids, oxidative stress, and energy metabolism in cardiac muscle. *Canadian Journal of Physiology and Pharmacology*, 239–245.

Feng, Z., Bai, L., Yan, J., Li, Y., Shen, W., Wang, Y., et al. (2011). Mitochondrial dynamic remodeling in strenuous exercise-induced muscle and mitochondrial dysfunction: Regulatory effects of hydroxytyrosol. *Free Radical Biology & Medicine*, 1437–1446.

Fernández-Jarne, E., Martínez-Losa, E., Prado-Santamaría, M., Brugarolas-Brufau, C., Serrano-Martínez, M., & Martínez-González, M. (2002). Risk of first non-fatal myocardial infarction negatively associated with olive oil consumption: a case-control study in Spain. *International Journal of Epidemiology*, 474-480.

Ferrara MD, L. A., Raimondi MD, A. S., d'Episcopo RD, L., Guida MD, L., Russo MS, A. D., & Marotta MD PhD, T. (2000). Olive Oil and Reduced Need for Antihypertensive Medications. *Archives of Internal Medicine*, 837-842.

Fito, M., Cladellas, M., de la Torre, R., Martıc, J., Alcantara, M., Pujadas-Bastardes, M., et al. (2005). Antioxidant effect of virgin olive oil in patients with stable coronary heart disease: a randomized, crossover, controlled, clinical trial. *Atherosclerosis*, 149–158.

Fito, M., Cladellas, M., de la Torre, R., Marti, J., Munoz, D., Schroder, H., et al. (2008). Anti-inflammatory effect of virgin olive oil in stable coronary disease patients: a randomized, crossover, controlled trial. *European Journal of Clinical Nutrition*, 570–574.

Fitó, M., de la Torre, R., Farré-Albaladejo, M., Khymenetz, O., Marrugat, J., & Covas, M.-I. (2007). Bioavailability and antioxidant effects of olive oil phenolic compounds in humans: a review. *ANN IST SUPER SANITÀ*, 375-381.

Ford, E. S. (2002). Does Exercise Reduce Inflammation? Physical Activity and C-Reactive Protein Among U.S. Adults. *Epidemiology*, 561-568.

Galli, C., & Visioli, F. (1999). Antioxidant and Other Activities of Phenolics in Olives/Olive Oil, Typical Components of the Mediterranean Diet. *Lipids*, S23-S26.

Gleeson, M. (2007). Immune function in sport and exercise. *Journal of Applied Physiology*, 693–699.

Gonzalez-Santiago, M., Martin-Bautista, E., Carrero, J., Fanolla, J., Baro, L., Bartolome, M., et al. (2006). One-month administration of hydroxytyrosol, a phenolic antioxidant present in olive oil, to hyperlipemic rabbits improves blood lipid profile, antioxidant status and reduces atherosclerosis development. *Atherosclerosis* (188), 35-42.

Gordon, M. H., Paiva-Martins, F., & Almeida, M. (2001). Antioxidant Activity of Hydroxytyrosol Acetate Compared with That of Other Olive Oil Polyphenols. *Journal of Agricultural and Food Chemistry*, 2480−2485.

Goto, C., Nishioka, K., Umemura, T., Jitsuiki, D., Sakagutchi, A., Kawamura, M., et al. (2007). Acute Moderate-Intensity Exercise Induces Vasodilation Through an Increase in Nitric Oxide Bioavailiability in Humans. *American Journal of Hypertension*, 825–830.

Granados-Principal, S., Quiles, J. L., Ramirez-Tortosa, C. L., Sanchez-Rovira, P., & Carmen, R.-T. M. (2010). Hydroxytyrosol: from laboratory investigations to future clinical trials. *Nutrition Reviews*, 191–206.

Halliwell, B., Aeschbach, R., Loliger, J., & Aruoma, O. I. (1995). The Characterization of Antioxidants. *Food and Chemical Toxicology*, 601-617.

Hao, J., Shen, W., Yu, G., Jia, H., Li, X., Feng, Z., et al. (2010). Hydroxytyrosol promotes mitochondrial biogenesis and mitochondrial function in 3T3-L1 adipocytes. *Journal of Nutritional Biochemistry* (21), 634-644.

Himmerich, H., Fulda, S., Linseisen, J., Seiler, H., Wolfram, G., Himmerich, S., et al. (2006). TNF-a, soluble TNF receptor and interleukin-6 plasma levels in the general population. *Eur. Cytokine Netw.*, 196-201.

Huertas, J., Martinez-Velasco, E., Ibanez, S., Lopez-Frias, M., Ochoa, J., Quiles, J., et al. (1999). Virgin olive oil and coenzyme Q10 protect heart mitochondria from peroxidative damage during aging. *BioFactors* (9), 337-343.

Kies, A. K., Rietjens, S. J., Haenen, G. R., & Bast, A. (2007). *Patent No. PCT/EP05/55987.* United States.

Leeuwenburg, C., & Heinecke, J. W. (2001). Oxidative Stress and Antioxidants in Exercise. *Current Medicinal Chemistry*, 829-838.

Leger, C., Carbonneau, M., Michel, F., Mas, E., Monnier, L., Cristol, J., et al. (2005). A thromboxane effect of a hydroxytyrosol-rich olive oil wastewater extract in patients with uncomplicated type I diabetes. *European Journal of Clinical Nutrition*, 727–730.

Liesen, H., Dufaux, B., & Hollmann, W. (1977). Modifications of Serum Glycoproteins the Days Following a Prolonged Physical Exercise and the Influence of Physical Training. *European Journal of Applied Physiology and Occupational Physiology*, 243-254.

Liu, J., Shen, W., Zhao, B., Wang, Y., Wertz, K., Weber, P., et al. (2009). Targeting mitochondrial biogenesis for preventing and treating insulin resistance in diabetes and obesity: Hope from natural mitochondrial nutrients. *Advanced Drug Delivery Reviews*, 1343–1352.

Liu, M.-L., Bergholm, R., Makimattila, S., Lahdenpera, S., Valkonen, M., Hilden, H., et al. (1999). A marathon run increases the susceptibility of LDL to oxidation in vitro and modifies plasma antioxidants. *Am J Physiol Endocrinol Metab*, E1083-E1091.

Manna, C., Galleti, P., Cucciolla, V., Montedoro, G., & Zappia, V. (1999). Olive oil hydroxytyrosol protects human erythrocytes against oxidative damages. *J Nutr Biochem* (10), 159-165.

Manna, C., Galletti, P., Cucciolla, V., Moltedo, O., Leone, A., & Zappia, V. (1997). The Protective Effect of the Olive Oil Polyphenol (3,4-Dihydroxyphenyl)-ethanol Counteracts Reactive Oxygen Metabolite–Induced Cytotoxicity in Caco-2 Cells. *The Journal of Nutrition*, 286-292.

Manna, C., Migliardi, V., Sannino, F., De Martino, A., & Capasso, R. (2005). Protective Effects of Synthetic Hydroxytyrosol Acetyl Derivatives against Oxidative Stress in Human Cells. *Journal of Agricultural and Food Chemistry*, 9602−9607.

Masella, R., Giovannini, C., Varì, R., Di Benedetto, R., Coni, E., Volpe, R., et al. (2001). Effects of Dietary Virgin Olive Oil Phenols on Low Density Lipoprotein Oxidation in Hyperlipidemic Patients. *Lipids*, 1195-1202.

Mastaloudis, A., Leonard, S. W., & Traber, M. G. (2001). Oxidative stress in athletes during extreme endurance exercise. *Free Radical Biology & Medicine*, 911-922.

Mataix, J., Battino, M., Ramirez-Tortosa, M. C., Bertoli, E., & Quiles, J. L. (2008). Virgin olive oil: a key healthy component of the Mediterranean diet. *Mediterr J Nutr Metab*, 69-75.

Mataix, J., Quiles, J. L., Huertas, J. R., Battino, M., & Manas, M. (1998). Tissue specific interactions of esercise, dietary fatty acids, and vitamin E in lipid peroxidation. *Free Radical Biology & Medicine, 24* (4), 511-521.

Mateos, R., Dominguez, M. M., Espartero, J. L., & Cert, A. (2003). Antioxidant Effect of Phenolic Compounds, alpha-Tocopherol, and Other Minor Components in Virgin Olive Oil. *Journal of Agricultural and Food Chemistry*, 7170−7175.

McDonald, S., Prenzler, P. D., Antolovich, M., & Robards, K. (2001). Phenolic content and antioxidant activity of olive extracts. *Food Chemistry*, 73-84 .

Minich, D. M., & Bland, J. S. (2008). Dietary management of the metabolic syndrome beyond macronutrients. *Nutrition Reviews, 66* (8), 429-444.

Miro-Casas, E., Covas, M.-I., Farre, M., Fito, M., Ortuno, J., Weinbrenner, T., et al. (2003). Hydroxytyrosol Disposition in Humans. *Clinical Chemistry*, 945–952.

Miro-Casas, E., Covas, M.-I., Fito, M., Farre-Albadalejo, M., Marrugat, J., & de la Torre, R. (2003). Tyrosol and hydroxytyrosol are absorbed from moderate and sustained doeses of virgin olive oil in humans. *European Journal of Clinical Nutrition* (57), 186-190.

Mizuhara, H., O'Neill, E., Seki, N., Ogawa, T., Kusunoki, C., Otsuka, K., et al. (1994). T Cell Activation-associated Hepatic Injury: Mediation by Tumor Necrosis Factors and Protection by Interleukin 6. *J. Exp. Med.*, 1529-1537.

Morillas-Ruiz, J., Villegas Garcia, J., Lopes, F., Vidal-Guevara, M., & Zafrilla, P. (2006). Effects of polyphenolic antioxidants on exercise-induced oxidative stress. *Clinical Nutrition* (25), 444-453.

Nieman, D. C., Dumke, C. L., Henson, D. A., McAnulty, S. R., Gross, S. J., & Lind, R. H. (2005). Muscle damage is linked to cytokine changes following a 160-km race. *Brain, Behavior, and Immunity*, 398-403.

Nieman, D. C., Henson, D. A., Smith, L. L., Utter, A. C., Vinci, D. M., Davis, J. M., et al. (2001). Cytokine changes after a marathon race. *Journal of Applied Physiology*, 109 – 114.

Owen, R. W., Giacosa, A., Hull, W. E., Haubner, R., Würtele, G., Spiegelhalder, B., et al. (2000). Olive-oil consumption and health: the possible role of antioxidants. *The Lancet: Oncology*, 107-112.

Owen, R., Giacosa, A., Hull, W., Haubner, R., Spiegelhalder, B., & Bartsch, H. (2000). The antioxidant/anticancer potential of phenolic compounds isolated from olive oil. *European Journal of Cancer*, 1235-1247.

Patrick, L., & Uzick, M. (2001). Cardiovascular Disease: C-Reactive Protein and the Inflammatory Disease Paradigm: HMG-CoA Reductase Inhibitors, alpha-Tocopherol, Red Yeast Rice, and Olive Oil Polyphenols. A Review of the Literature. *Alternative Medicine Review*, 248-271.

Pellegrini, N., Serafini, M., Colombi, B., Del Rio, D., Salvatore, S., Bianchi, M., et al. (2003). Total Antioxidant Capacity of Plant Foods, Beverages and Oils Consumed in Italy Assessed by Three Different In Vitro Assays. *The Journal of Nutrition*, 2812-2819.

Petersen, A. M., & Pedersen, B. K. (2005). The anti-inflammatory effect of exercise. *Journal of Applied Physiology*, 1154 –1162.

Petroni, A., Blasevich, M., Salami, M., Papini, N., Montedoro, G. F., & Galli, C. (1995). Inhibition of Platelet Aggregation and Eicosanoid Production by Phenolic Components of Olive Oil. *Thrombosis Research*, 151-160.

Powers, S. K., & Jackson, M. J. (2008). Exercise-Induced Oxidative Stress: Cellular Mechanisms and Impact on Muscle Force Production. *Physiological Reviews*, 1243–1276.

Powers, S. K., DeRuisseau, K. C., Quindry, J., & Hamilton, K. L. (2004). Dietary antioxidants and exercise. In R. Maughan, L. Burke, & E. Coyle (Ed.), *Food, Nutrition and Sports Performance II* (pp. 130-152). London and New York: Routledge, Taylor & Francis Group.

Psaltopoulou, T., Naska, A., Orfanos, P., Trichopoulos, D., Mountokalakis, T., & Trichopoulou, A. (2004). Olive oil, the Meditteranean diet, and arterial blood pressure: the Greek European Prospective Investigation into Cancer and Nutrition (EPIC) study. *The American Journal of Clinical Nutritioin* (80), 1012-1018.

Quiles, J. L., Farquharson, A. J., Simpson, D. K., Grant, I., & Wahle, K. W. (2002). Olive oil phenolics: effects on DNA oxidation and redox enzyme mRNA in prostate cells . *British Journal of Nutrition*, 225-234.

Quindry, J. C., Stone, W. L., King, J., & Broeder, C. E. (2003). The Effects of Acute Exercise on Neutrophils and Plasma Oxidative Stress. *Medicine & Science in Sports & Exercise*, 1139-1145.

Raederstorff, D. (2009). Antioxidant activity of olive polyphenols in humans: a review. *International Journal for Vitamin and Nutrition Research*, 1-15.

Rietjens, S. J., Bast, A., & Haenen, G. R. (2007). New Insights into Controversies on the Antioxidant Potential of the Olive Oil Antioxidant Hydroxytyrosol. *Journal of Agricultural and Food Chemistry*, 7609−7614.

Ruano, J., Lopez-Miranda, J., de le Torre, R., Delgado-Lista, J., Fernandez, J., Caballero, J., et al. (2007). Intake of phenol-rich virgin olive oil improves the postprandial prothrombotic profile in hypercholesterolemic patients. *The American Journal of Clinical Nutrition* (86), 31-346.

Schwedhelm, E., Maas, R., Troost, R., & Boger, R. H. (2003). Clinical Pharmacokinetics of Antioxidants and Their Impact on Systemic Oxidative Stress. *Clinical Pharmacokinetics*, 437-459.

Smith, M. J., Dykes, B. R., Douglas, M. J., Krishnaswamy, M. G., & Berk, M. S. (1999). Long-term Exercise and Atherogenic Activity of Blood Mononuclear Cells in Persons at Risk of Developing Ischemic Heart Disease. *Journal of the American Medical Association*, 1722-1727.

Sporer, B., & McKenzie, D. C. (2007). Reproducibility of a Laboratory Based 20-km Time Trial Evaluation in Competitive Cyclists Using the Velotron Pro Ergometer. *International Journal of Sports Medicine* (28), 940-944.

Suzuki, K., Yamada, M., Kurakake, S., Okamura, N., Yamaya, K., Liu, Q., et al. (2000). Circulating cytokines and hormones with immunosuppressive but neutrophil-

priming potentials rise after endurance exercise in humans. *European Journal of Applied Physiology*, 281-287.

Teixeira, V. H., Valente, H. F., Casal, S. I., Marques, A. F., & Moreira, P. A. (2009). Antioxidants Do Not Prevent Postexercise Peroxidation and May Delay Muscle Recovery. *Medicine & Science in Sports & Exercise*, 1752-1760.

Tietz, N. (Ed.). (1995). "Clinical Guide to Laboratory Tests". *(19106), 3rd*. Philadelphia, PA: W.B. Saunders Company.

Tuck, K. L., & Hayball, P. J. (2002). Major phenolic compounds in olive oil: metabolism and health effects. *The Journal of Nutritional Biochemistry* (13), 636-644.

Urso, M. L., & Clarkson, P. M. (2003). Oxidative stress, exercise, and antioxidant supplementation. *Toxicology*, 41-54.

Vassilakopoulos, T., Karatza, M.-H., Katsaounou, P., Kollintza, A., Zakynthinos, S., & Roussos, C. (2003). Antioxidants attenuate the plasma cytokine response to exercise in humans. *J Appl Physiol*, 1025–1032.

Vazquez-Velasco, M., Diaz, L. E., Lucas, R., Gomez-Martinez, S., Bastida, S., Marcos, A., et al. (2010). Effects of hydroxytyrosol-enriched sunflower oil consumption on CVD risk factors. *British Journal of Nutrition*, 1-5.

Viguie, C. A., Frei, B., Shigenaga, M. K., Ames, B. N., Packer, L., & Brooks, G. A. (1993). Antioxidant status and indexes of oxidative stress during consecutive days of exercise . *Journal of Applied Physiology*, 566-572.

Vincent, H., & Taylor, A. (2006). Biomarkers and potential mechanisms of obesity-induced oxidant stress in humans. *International Journal of Obesity*, 400–418.

Visioli, F., & Galli, C. (1998). Olive Oil Phenols and Their Potential Effects on Human Health. *Journal of Agricultural and Food Chemistry*, 4292−4296.

Visioli, F., Caruso, D., Galli, C., Viappiani, S., Galli, G., & Sala, A. (2000). Olive Oils Rich in Natural Catecholic Phenols Decrease Isoprostane Excretion in Humans. *Biochemical and Biophysical Research Communications*, 797–799.

Visioli, F., Poli, A., & Galli, C. (2001). Antioxidant and Other Biological Activities of Phenols from Olives and Olive Oil. *Medicinal Research Reviews*, 65-75.

Wang, L., Geng, C., Jiang, L., Gong, D., Liu, D., Yoshimura, H., et al. (2008). The anti-atherosclerotic effect of olive leaf extract is related to suppressed inflammatory response in rabbits with experimental atherosclerosis. *European Journal of Nutrition*, 235–243.

Wang, Z., Ciabattoni, G., Créminon, C., Lawson, J., Fitzgerals, G. A., Patrono, C., et al. (1995). Immunological characterization of urinary 8-epi-prostaglandin F2 alpha excretion in man. *The Journal of Pharmocology*, 94-100.

Weinbrenner, T., Fito, M., de la Torre, R., Saez, G. T., Rijken, P., Tormos, C., et al. (2004). Olive Oils High in Phenolic Compounds Modulate Oxidative/Antioxidative Status in Men. *The Journal of Nutrition*, 2314-2321.

VITA

Ashlee Danielle Simpson was born in Amarillo, TX. After graduating from Randall High School, Amarillo, TX, in 2004, she attended Wayland Baptist University for one semester. She then transferred to West Texas A&M University in Canyon, TX where she graduated *magna cum laude* with a Bachelor of Science in Sports and Exercise Science and Biology in May 2009. That fall she entered the Graduate School at The University of Texas at Austin to pursue a Master of Science degree in Exercise Physiology.

Permanent Email Address: adsimpson2@gmail.com

This thesis was typed by the author.